INFLUENCE OF SOLVENT ON THE RING-CHAIN HYDROLYSIS EQUILIBRIUM OF ANABASEINE AND SYNTHESES OF ANABASEINE AND NICOTINE ANALOGUES

BY

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

INFLUENCE OF SOLVENT ON THE RING-CHAIN HYDROLYSIS EQUILIBRIUM OF ANABASEINE AND SYNTHESES OF ANABASEINE AND NICOTINE ANALOGUES

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December, 1990

Chairman: John A. Zoltewicz
Major Department: Chemistry

Anabaseine (3,4,5,6-tetrahydro-2,3'-bipyridine), a marine toxin produced by some species of Nemertines, is hydrolyzed in aqueous solution to form an open-chain amino ketone. This toxin, used by the worms to paralyze their prey, is active on vertebrate cholinergic nicotinic receptors as well as on arthropod receptors. In addition to anabaseine, other species of nemertines produce related pyridine alkaloids such as 2,3'-bipyridine and nemertelline, a tetrapyridyl. These natural products and analogues are useful model compounds for characterizing the binding sites of nicotinic receptors.

Because of the biological significance of anabaseine and the lack of both a qualitative and a quantitative

understanding of the hydrolysis equilibrium for cyclic imines, we performed a systematic investigation of the hydrolysis equilibrium of anabaseine. The hydrolysis reaction was first studied in water since biological systems are largely aqueous. The relative concentrations of amino ketone and cyclic imine in aqueous solutions over a pD range of about 2-10 were measured using proton NMR. The resulting pD-hydrolysis profile was computer fit using a model for the equilibria to arrive at values for the hydrolysis equilibrium constant (K_H) and dissociation constants (K_a) for the basic nitrogen atoms. We demonstrated the general utility of this approach by calculating equilibrium constants from data for other pH-dependent equilibria which appeared in the literature.

Because binding sites may be located in hydrophobic pockets of receptors, the compositions of equilibrium mixtures of anabaseine and of N-methylanabaseine (1-methyl-3,4,5,6-tetrahydro-2,3'-bipyridinium ion) were measured using proton NMR in nonaqueous systems (CD_3OD and DMSO-d_6). A general survey of solvent effects is presented along with a quantitative study in water-methanol and water-DMSO mixtures. The compositions of solutions of anabaseine and of N-methylanabaseine in water-methanol mixtures can be described by linear free energy relationships.

Analogues of nicotine, a known agonist on nicotinic receptors, anabaseine, and 2,3'-bipyridine were prepared for biological testing.

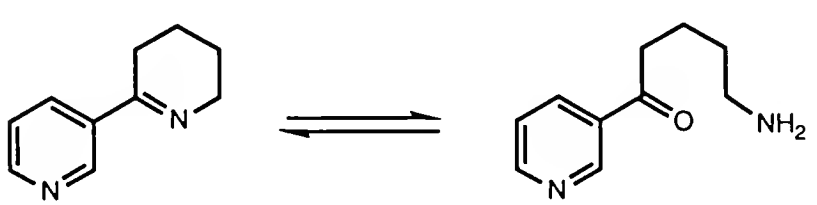
Finally, methods which could be used to investigate the nature of binding environments were explored using a micellar model system. Proton chemical shifts and longitudinal relaxation times were used to probe the binding environment of substrates in SDS micelles.

CHAPTER 1
INTRODUCTION

General Overview

Anabaseine (1-1, 3,4,5,6-tetrahydro-2,3'-bipyridine) is present in the venom of some species of nemertines, a phylum of carnivorous marine worms [1 - 3]. The venom is used to paralyze the worms' prey and possibly to repel predators. Anabaseine is of pharmacological interest because of its activity as an agonist on cholinergic nicotinic receptors in the central and peripheral nervous systems. Its activity is comparable to that of nicotine.

The structure of anabaseine consists of a 1-piperideine ring attached at its 2-position to the 3-position of a pyridine ring. Imines are water labile and can be hydrolyzed to give an amine and ketone or aldehyde. In aqueous solution anabaseine is hydrolyzed to give an open-chain amino ketone which is in equilibrium with the cyclic imine (eq 1-1) [4]. Both of these structures contain basic nitrogen sites. Thus, species which vary in protonation state may be present in an equilibrium mixture also.



(1-1)

The possibility of several forms of anabaseine existing in aqueous solutions raises many interesting questions concerning its biological activity. Which form is responsible for nicotinic activity? Why is anabaseine not toxic to the worm? Perhaps, the worm stores the toxin in an inactive form and then changes the medium to convert the toxin to an active form when it stings its prey [3].

Anabaseine is also of biological significance due to its activity on nicotinic receptors. Various types of nicotinic receptors which respond differently to known agonists and antagonists exist within the many parts of the central and peripheral nervous systems. While the gross structures of some of these receptors are known, their binding sites have not been well characterized. Anabaseine and other structurally related model compounds can be used to help define the nature of the binding sites of nicotinic receptors.

While the hydrolysis of acyclic imines has been extensively studied [5 - 7], comparatively little work has been done on the hydrolysis equilibria of cyclic imines. Because of the biological significance of anabaseine and the lack of both a qualitative and a quantitative understanding of the hydrolysis equilibria for cyclic imines in general, we undertook a systematic investigation of the hydrolysis equilibrium of anabaseine. Our study began with a quantitative determination of the equilibrium compositions of aqueous solutions of anabaseine from which we were able to

derive an equilibrium constant for hydrolysis (K_H) and pK_a values. This was followed by a general survey of solvent effects on the position of the equilibrium and finally a quantitative study of the system in mixed solvents which contained water. In addition to the equilibrium studies, model compounds were prepared for biological testing. These model compounds were designed with two goals in mind: (1) to help determine which form of anabaseine was biologically active and (2) to aid in the characterization of the binding sites of nicotinic receptors and the determination of those structural features that would render a molecule active. Questions concerning the environment of the binding site at nicotinic receptors sparked our interest in methods that could be used to investigate binding environments. In order to explore the possibilities, model studies were done on the binding of substrates to micelles. We used proton NMR techniques to gain information about the environments of different substrates when bound to SDS micelles.

Brief Summary of Investigations by Chapter

Because biological systems are largely aqueous, the hydrolysis reaction of anabaseine was first investigated in water to determine how the equilibrium composition changed with pH. Both proton NMR and UV measurements were made on aqueous solutions of anabaseine. The results of these measurements appear in Chapter 2. The NMR measurements over

a pD range of about 2 to 10 showed that anabaseine exists as a mixture of four possible species depending on the solution pD: (1) cyclic imine free base (1-1), (2) cyclic iminium ion (1-2), (3) monoprotonated amino ketone (1-3) where only the amino group is protonated, and (4) diprotonated amino ketone (1-4) where both the pyridine and amino nitrogen atoms were protonated (Figure 1-1).

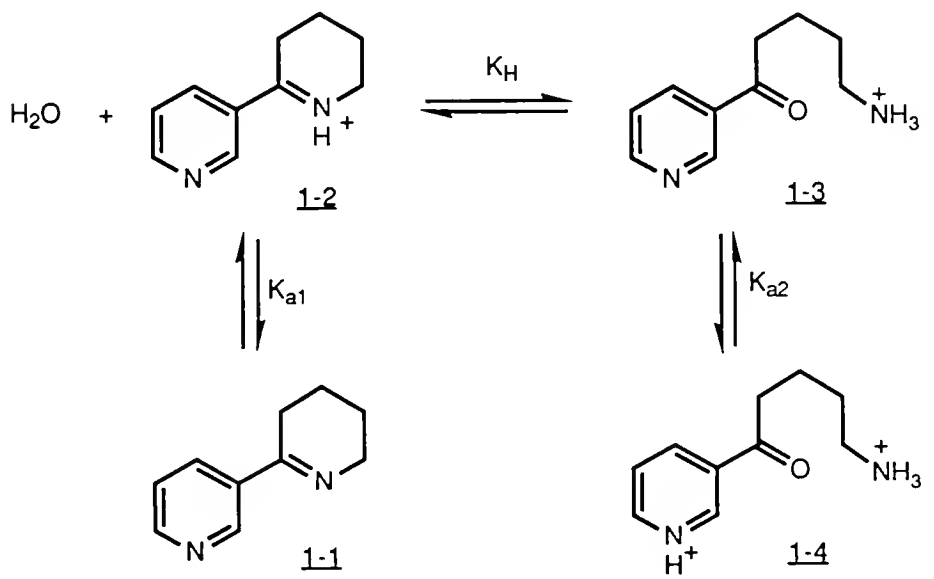


Figure 1-1. Anabaseine equilibria in aqueous solution.

These NMR measurements made possible a quantitative determination of the effects of pD on the equilibrium concentrations of each component in heavy water. The NMR data describing the fraction of cyclic imine over the pH range of 2-10 were computer simulated using the model for the equilibria in Figure 1-1 and iterative nonlinear regression analysis [4]. The computer fit of the data yielded a

hydrolysis equilibrium constant (K_H) and pK_a values for anabaseine.

Because each UV spectrum is the sum of the spectra of all the species present in equilibrium, the UV spectra do not contain as much information as the NMR spectra. Both the molar absorptivities of each species and the amount of each present are unknown; this makes analysis more complicated. The results obtained from UV measurements supported the results obtained from NMR measurements although a unique fit of the data was not obtained using the model in Figure 1-1 and the nonlinear regression program.

The method used to simulate the pD-hydrolysis profile for anabaseine and to obtain equilibrium constants is general. The utility of this method was demonstrated by applying it to some other examples of pH-dependent equilibria which appeared in the literature.

Brandänge and coworkers presented data for the pH-dependent hydrolysis of myosmine, a minor tobacco alkaloid, and other structurally related imines (Figure 1-2) which were based on NMR measurements in aqueous solutions [8, 9]. Myosmine is structurally very similar to anabaseine but contains a 5-membered 1-pyrroline ring in place of the 1-piperideine ring of anabaseine. A quantitative analysis of equilibrium constants for these compounds was not given by Brandänge, however. We devised models for these equilibria and used our computer simulation to fit the data and extract the appropriate equilibrium constants [4].

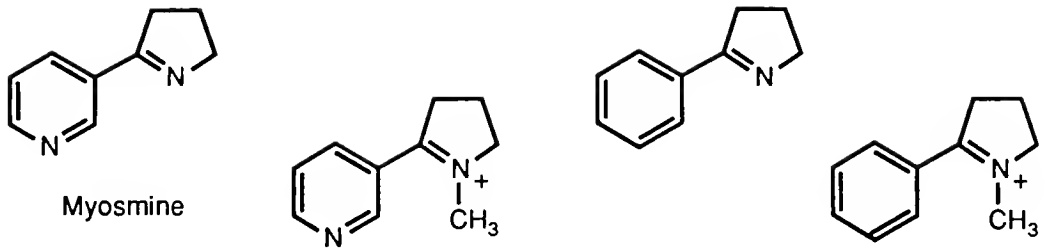
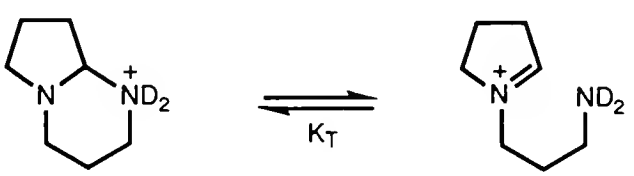


Figure 1-2. Myosmine and analogues studied by Brändange and coworkers.

The computer simulation of pH-dependent equilibria is not limited to the hydrolysis of cyclic imines. NMR measurements in aqueous solution were reported in the literature for a pH-dependent tautomeric equilibrium (eq 1-2) between a protonated bicyclic aminal, octahydropyrrolo[1,2-a]pyrimidinium ion (1-5), and an open-chain monocyclic amino pyrrolinium ion, 3-aminopropyl-1-pyrrolinium ion (1-6) [10]. We were also able to fit these data by computer and derive equilibrium constants [11]. The results of these fits appear in Chapter 3.



(1-2)

1-5 1-6

Our studies of the anabaseine equilibrium also included nonaqueous systems. Although biological systems are largely aqueous, the binding site at nicotinic receptors for anabaseine and other agonists may be located in a hydrophobic pocket of the proteins that form the receptor.

A hydrophobic environment at the receptor site may have different effects on the hydrolysis equilibrium for anabaseine than are observed in aqueous solution. For this reason, the influence of nonaqueous systems on the equilibrium composition of anabaseine was investigated. N-Methylanabaseine, a derivative of anabaseine which possesses a methyl substituent on the imine nitrogen, was prepared so that comparisons could be made between the effects of the substituent on the position of the equilibrium and the biological activity. Because data on biological activity were obtained by others, such information is not reported here. The compositions of anabaseine and N-methylanabaseine solutions in DMSO-d_6 and in methanol-d_4 were measured using proton NMR and compared with those in D_2O [12]. These solutions contained at least one equivalent of water so that a hydrolysis equilibrium could be established. Results of these measurements are reported in Chapter 4. The amounts of amino ketone and cyclic imine observed in the nonaqueous systems did not change dramatically from those observed in aqueous systems in spite of the fact that the concentration of water present was dramatically decreased.

The equilibrium compositions of anabaseine and N-methylanabaseine were measured in water-DMSO and water-methanol mixtures also. As the amount of water was increased in the mixed solvents, the equilibria shifted to favor ketone as expected from mass action considerations. The equilibrium compositions of both compounds in methanol-water mixtures can

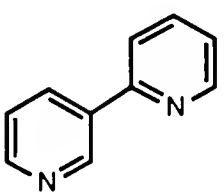
be quantitatively described by a linear free energy relationship. These results are discussed in Chapter 4.

The methyl substituent of N-methylanabaseine shifts the position of the equilibrium, largely for steric reasons, so that more of the amino ketone is present in these aqueous solutions than in solutions of anabaseine. These results stand in marked contrast to those pertaining to the effect of methyl substitution on the position of equilibrium of myosmine and its N-methyl derivative. Here methyl substitution leads to a decrease in the amount of amino ketone.

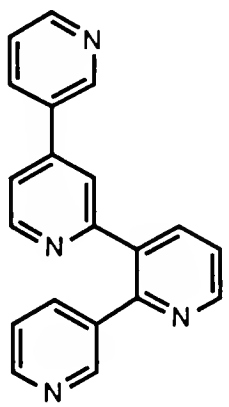
In addition to anabaseine, other species of nemertine worms produce other alkaloids. Two such compounds are 2,3'-bipyridine and nemertelline, a tetrapyridine [13]. Another compound which has not yet been identified gives a mass spectrum which is consistent with a methyl substituted bipyridine [Kem, unpublished results]. While 2,3'-bipyridine is not very active in vertebrates, it is quite active in crustaceans. Nemertelline does not appear to be active in either vertebrates or arthropods and may have some other function such as a chemical attractant or repellant. A general method for the preparation of bi- and polypyridines was desired both for identification of natural products and to produce greater quantities of these compounds for biological testing.

Many methods of coupling aryl and alkenyl halides with aryl stannanes, boranes and boronic acids using a Pd (0)

catalyst have been reported [14 - 19]. Palladium catalyzed cross-coupling of pyridyl halides with pyridyl boranes was used to prepare substituted bipyridines and this approach is discussed in Chapter 6. Bipyridines with different substituents can easily be prepared using this method allowing comparisons to be made between substitution patterns and biological activity.



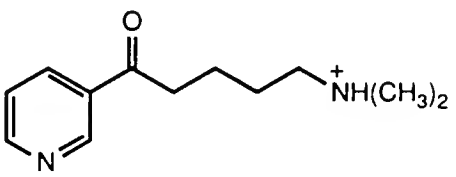
2,3'-Bipyridine



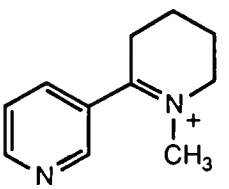
Nemertelline

The pharmacological properties of these natural products and derivatives render them useful for the study of nicotinic receptors. Comparisons of biological activities with differences in the structures and the properties of these compounds will aid in the characterization of the nature of the binding sites of nicotinic receptors within different parts of the nervous system. Characterization of arthropod receptors may aid in the development of insecticides and other forms of pest control. With this in mind, structural

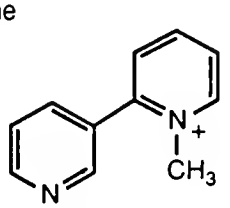
analogs of anabaseine, nicotine, and 2,3'-bipyridine were prepared. These compounds are shown in Figure 1-3 and their syntheses are discussed in Chapters 5 and 6.



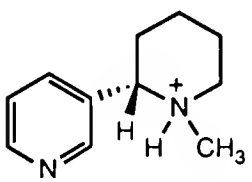
Dimethylamino Analogue of Anabaseine



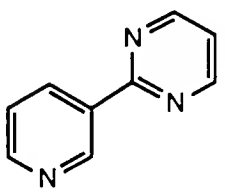
N-Methylanabaseine



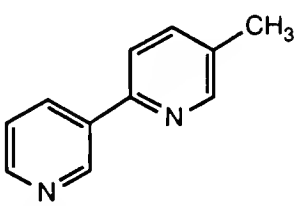
1-Methyl-2,3'-bipyridinium Ion



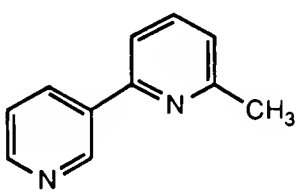
(S)-N-Methylanabasine Monocation



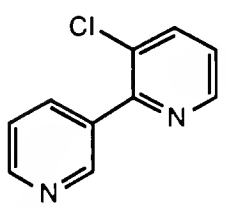
2-(3-Pyridyl)pyrimidine



5-Methyl-2,3'-bipyridine



6-Methyl-2,3'-bipyridine



3-Chloro-2,3'-bipyridine

Figure 1-3. Analogues of anabaseine, nicotine and 2,3'-bipyridine.

In the course of preparing 1-methyl-2,3'-bipyridinium ion, interesting differences in the proton NMR spectra of mono- and di-N-methylated 2,3'-bipyridines were observed. These differences provide information about the angles

between the planes of the rings in these bipyridinium ions. The chemistry of some diquaternized 2,3'-bipyridines also proved interesting. Meisenheimer-type σ complexes were observed on reaction of methoxide ion with these diquaternary salts. These results and the synthesis of 1-methyl-2,3'-bipyridinium ion are presented in Chapter 5.

In our studies of anabaseine, we became interested in methods that could be used to determine the type of environment of a binding site. Because biological systems are so complex, we explored some possibilities in model systems as a first step. We chose to investigate the environment of compounds which are solubilized by micelles. Micelles serve as primitive models for membranes and lipid bilayers and are composed of surfactants which have a polar head group and a nonpolar tail [20, 21]. In aqueous solution, micelles are formed when surfactants aggregate so that the hydrophobic portions of the molecules interact with each other and the hydrophilic portions of the molecule interact with water. Aqueous micellar solutions are able to solubilize hydrophobic molecules. For this reason, micelles are useful in reaction media and for many other applications.

Although micelles have been extensively studied many questions remained to be answered. There is still much disagreement over their exact structure, the degree of water penetration, and the structure of substrates when bound within micelles [20, 22 - 26]. We used proton NMR techniques to investigate the environment of substrates when they bind

to micelles. The substrates studied include adenosine, 2',3'-isopropylidene adenosine, and 1-methyl-4,4'-bipyridinium iodide. Micellar solutions of sodium dodecylsulfate (SDS) were used because the properties of SDS have been well characterized. The results of these studies appear in Chapter 7.

Finally, a detailed description of all experimental procedures is given in Chapter 8.

CHAPTER 2
QUANTITATIVE DETERMINATION OF THE RING-CHAIN HYDROLYSIS EQUILIBRIUM CONSTANT FOR ANABASEINE

Introduction

As discussed in Chapter 1, the structure of anabaseine consists of a 1-piperideine ring attached at the 2-position to a 3-pyridyl ring. The cyclic imine is water labile and is hydrolyzed in aqueous solution to produce an open-chain amino ketone.

The hydrolysis reaction in aqueous solution has been investigated using proton NMR and UV spectroscopy. The components present in aqueous solution over a pH range of 2-10 have been identified and for the first time the various equilibria associated with the hydrolysis have been described quantitatively [4]. Figure 2-1 shows the components (2-1 - 2-4) which exist in equilibrium; the relative amounts of each are dependent on pH.

Synthetic anabaseine used in this study was synthesized[1] by a previously reported method. In Chapter 6, an improved synthesis is discussed.

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[1] Prepared by Dr. William R. Kem.

Results

NMR Measurements of Aqueous Anabaseine Solutions

The hydrolytic equilibrium for anabaseine in D_2O was examined with the aid of proton NMR at 22 ± 1 °C at an ionic strength of 0.6 ± 0.08 M. The hydrolysis reaction was complete in the 5-10 minutes it took to prepare the sample and record the spectrum. Found were the cyclic imine 2-1 and its conjugate acid 2-2 and the hydrolysis product, the open-chain amino ketone 2-3 as well as its conjugate acid 2-4 (Figure 2-1). Examples of spectra at high, neutral and low pD's appear in Figures 2-2 and 2-3. These spectra illustrate the changes in the ratios of the two forms as evidenced by the changes in relative intensities of the peaks and the changes in the protonation states as evidenced by the changes in chemical shifts.

The samples were stable; after standing in a refrigerator for 9 months there was no evidence of change in the NMR spectrum. Some yellowing did occur, however. While the tetrahydropyridine ring in 2-1 may undergo dimerization and cyclic trimerization [27, 28], our solutions evidently were too dilute (0.008-0.018 M) for this to be an important side-reaction.

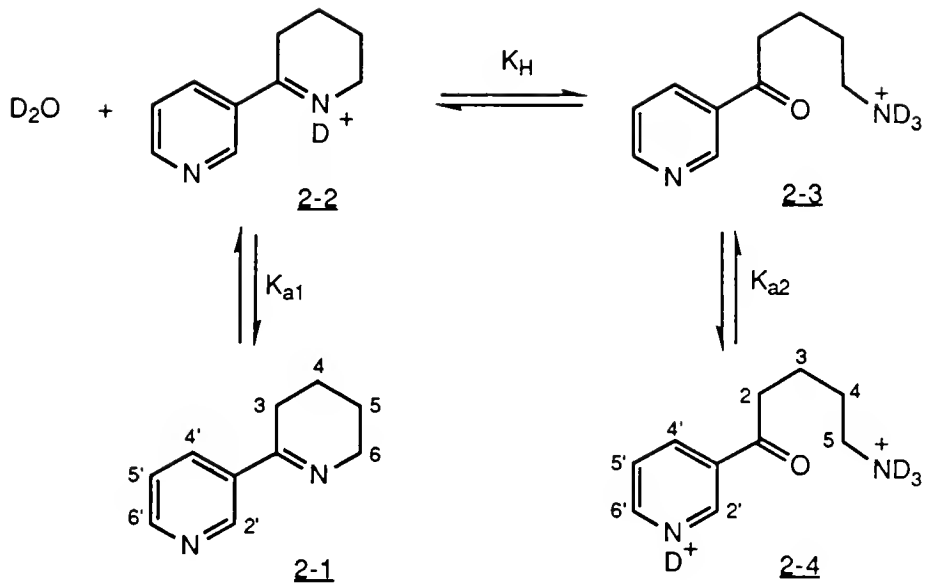


Figure 2-1. Model for anabaseine equilibria in aqueous solution.

Assignment of structures and composition in aqueous solutions

Both the high and low field portions of the spectra were used to assign structures. The high field NMR signals were attributed to cyclic and open-chain compounds using the chemical shifts of the methylene protons next to the nitrogen atom. Assignments rest on (a) the relative chemical shifts of these signals and (b) the influence of pD on their positions. For equivalent states of protonation, an sp^2 hybridized nitrogen atom is expected to be more deshielding than one that is sp^3 hybridized. Moreover, the amino group of the open-chain ketone of 2-3 is likely to remain largely as its conjugate acid because of its basicity and hence be invariant in shift over the pD range of our studies (2-10). But the position of the signal due to the less basic imine

Figure 2-2. ^{1}H NMR of anabaseine in D_2O at 22 °C and 0.6 M ionic strength at (a) pD 6.59 and (b) pD 8.65.

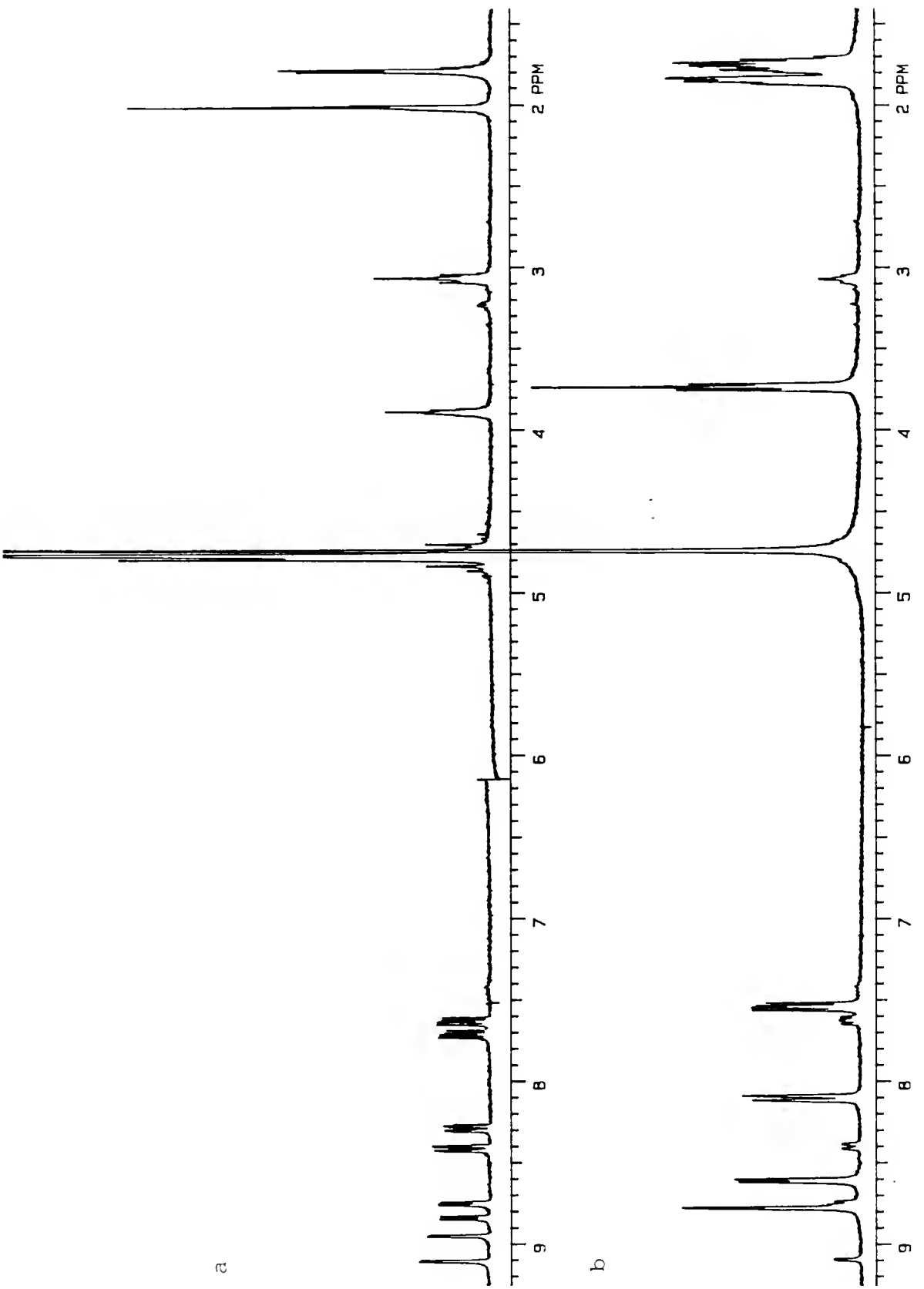
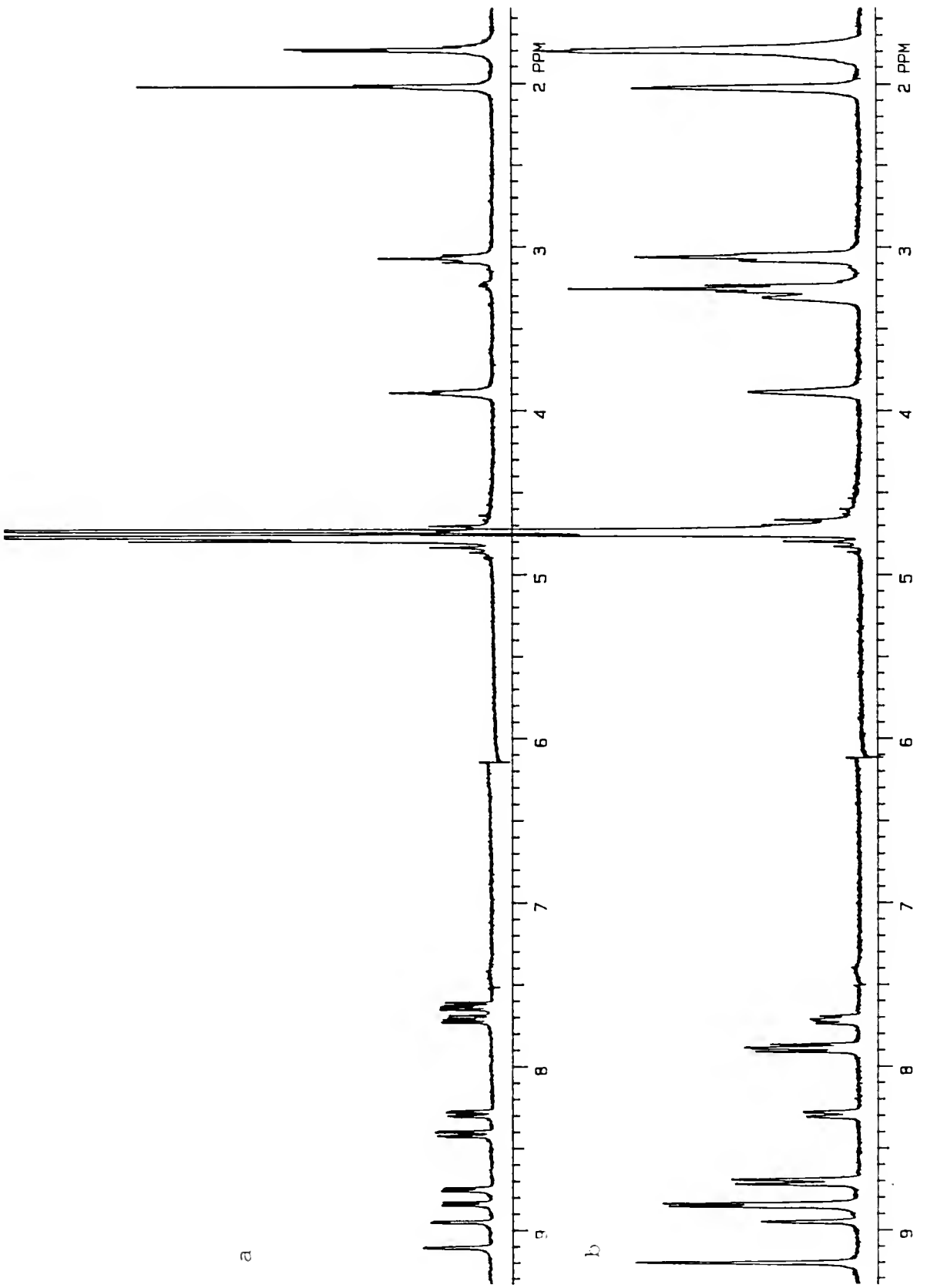

a
b
9
8
7
6
5
4
3
2 PPM
9
8
7
6
5
4
3
2 PPM

Figure 2-3. ^{1}H NMR of anabaseine in D_2O at 22 ^{o}C and 0.6 M ionic strength at (a) pD 6.59 and (b) pD 4.11.


a
b
2 PPM
2 PPM

2-1 will change as the pD of the medium is varied and the ratio of base and its conjugate acid changes. Therefore, the signal at δ 3.07 $\pm$ 0.01 which remained constant was assigned to the open chain amino ketone and that which varied from δ 3.72 to 3.89 to the cyclic imine (Figure 2-2). The ratio of the two forms was easily obtained by integration of these signals. Other signals associated with these two forms can also be assigned based on their relative intensities.

Shifts due to the CH_2CH_2 unit of the open and ring-closed forms overlapped and could not be used to determine structures. The protons next to the carbonyl group and adjacent to the imine carbon were replaced by deuterium. For example, at high pD little of these signals remained by the time the first spectrum was obtained. Even an acidic sample (pD 3.64), where isotope exchange was much slower, demonstrated deuterium incorporation over a period of a day at room temperature.

These structural assignments also are supported by a consideration of the chemical shifts at low field associated with the pyridine ring. For example, the signal for H-2 of the pyridine ring of one component (imine) moves to lower field (δ 8.75-8.95) over the pD range 9.57 to 6.59 and then remains constant. However, H-2 for the other component (ketone) was essentially constant at δ 9.10 from pD 9.57 to 6.24 and then gradually moved to lower field as the solution was made more acidic, reaching a final value of δ 9.34 at pD 2.20 (Figures 2-2 and 2-3).

The variation in the shifts of the pyridine protons at high pD can be attributed to changes in protonation of the cyclic imine while movement of the signals at low pD can be associated with changes in protonation of the pyridine of the acyclic ketone. The imine is more basic than the pyridine and its protonation will influence the chemical shifts of the attached pyridine ring. Since the nitrogen atom in the pyridine ring of the ketone is less basic than the imine, it will only undergo protonation and associated deshielding in more acidic media. The pyridine ring of the imine is likely to be less basic than that of the ketone due to the proximity and electron withdrawing effects of the conjugated iminium ion. Significant protonation of this ring is not observed. Figure 2-4 graphically reports our findings concerning the influence of solution acidity on the equilibrium composition of anabaseine during hydrolysis. The data are given in terms of the fraction of the total amount of substrate present as cyclic imine rather than as a ratio of two forms. This fraction varies from 0 to 1 and therefore is more easily understood. At high acidity (pD 2) nearly all the substrate is present in the open-chain form. As the solution is made more basic, the concentration of the imine increases and the amino ketone decreases. From about pD 5 to 7, the composition of the mixture is approximately constant. There is a sharp increase in the amount of cyclic imine as the solution is made still more alkaline until finally only cyclic imine is present at about pD 9.5.

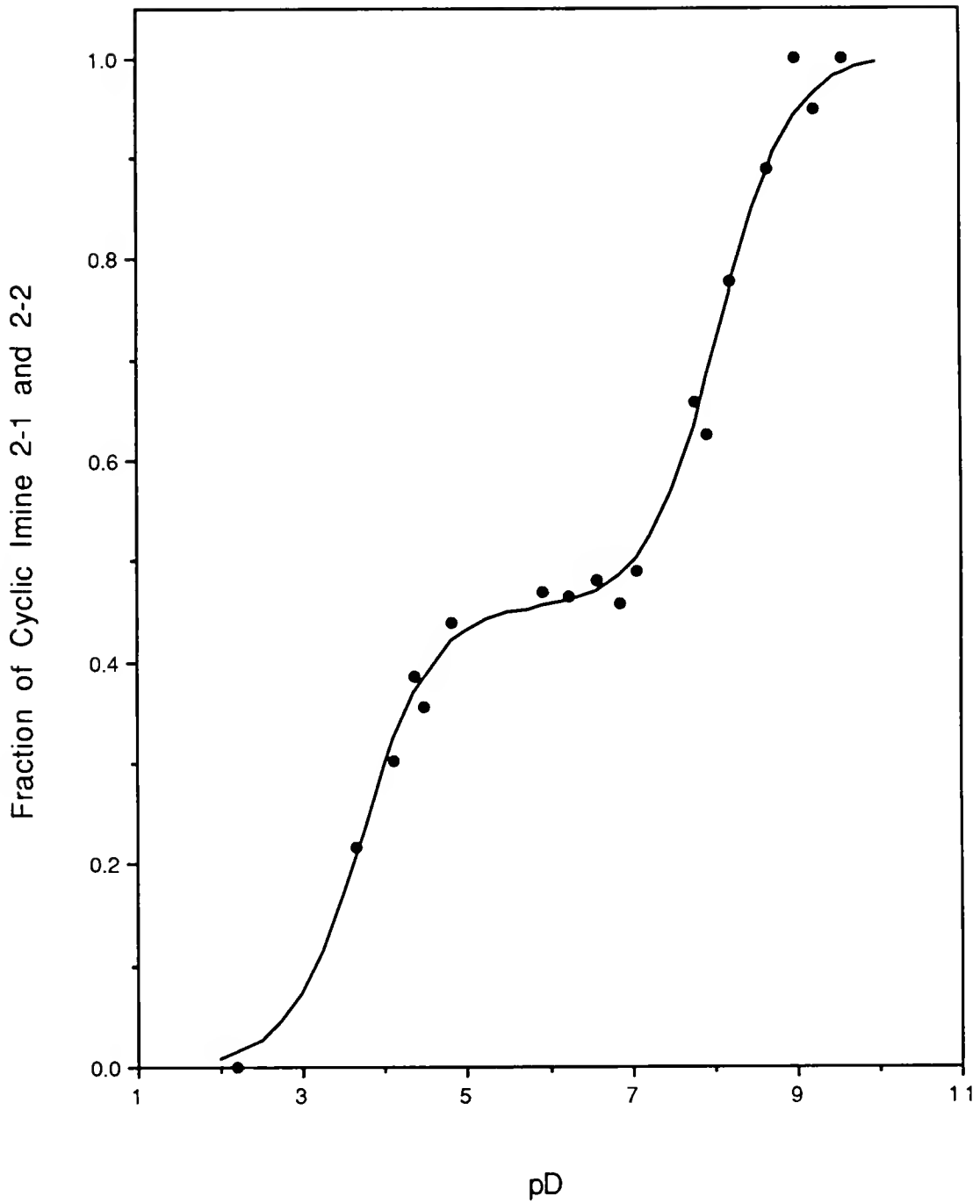


Figure 2-4. A titration curve describing the hydrolysis of anabaseine (2-1) in D_2O at 22 °C and 0.6 M ionic strength. The dark circles are the experimental values expressing the fractional amount of the total mixture existing as 2-1 and 2-2. The solid line was calculated using eq 2-3 and the values for the equilibrium constants in Table 2-2.

Calculation of pK_a values from pD dependent chemical shifts

Those proton signals that show chemical shift changes with pD provide information about pK_a values for the basic nitrogen sites. The observed chemical shift (δ_{obs}) at any pD is the population weighted average of the chemical shifts of the unprotonated (δ_N) and protonated (δ_{NH}) forms which can be expressed by eq 2-1.

$$\delta_{obs} = \delta_N \times \frac{K_a}{K_a + [D^+]} + \delta_{NH} \times \frac{[D^+]}{K_a + [D^+]} \qquad (2\text{-}1)$$

The fractional amount of free base is given by the expression $K_a/(K_a + [D^+])$ and the fraction of protonated substrate by $[D^+]/(K_a + [D^+])$.

Values of the dissociation constant (K_a) for the conjugate acid, 2-2, of the imine nitrogen of 2-1 (K_{a1}) and the chemical shifts δ_N and δ_{NH} were obtained by a nonlinear, iterative regression analysis using eq 2-1 to fit the observed chemical shifts associated with the signals of the methylene protons α to the nitrogen atom. Observed chemical shifts are given in Table 2-1 and values obtained from the computer fit are recorded in Table 2-2.

Similarly, the observed chemical shifts of each of the pyridine protons in the open-chain amino ketone is a weighted average of the chemical shifts of the protonated and unprotonated forms. From a consideration of the pK_a values of model compounds such as 2,3'-bipyridine (pK_a's 1.5, 4.4) [29]

Table 2-1. Observed Chemical Shifts for H6 of the Cyclic Imine Form of Anabaseine in D_2O at 22 °C and 0.6 M Ionic Strength.

pD	Chemical Shift / ppm[a]
8.65	3.738
8.20	3.769
7.93	3.819
7.77	3.804
7.07	3.868
6.88	3.883
6.59	3.893

[a]TSP was used as an internal standard.

we assume that protonation of the pyridine ring conjugated with the positively charged iminium ion will not be important over the pD range where the pyridine ring of the ketone is protonated. Therefore, the dissociation constant of the pyridinium ion in the cyclic imine was neglected and the changes in the chemical shifts of the pyridine protons at low pD were assigned to the open-chain ketone 2-4. Its K_{a2} was calculated by the nonlinear regression technique using the data for the H2', H4' and H5' protons separately and eq 2-1. Observed shifts for the pyridine protons appear in Table 2-3 and results from the computer fit are given in Table 2-2 and are within the usual agreement found for an NMR method [30]. Our pK_a value of 3.36 (3.86-0.50) corrected for the solvent

Table 2-2. Equilibrium Constants and Chemical Shifts for the Compounds in Figure 2-1 in D_2O at 22 ± 1 °C and 0.6 M Ionic Strength

10^8 K_{a1}	10^4 K_{a2}	K_H	δ_N	δ_{NH}	Eq.[a]
1.29 (0.51)			3.71 (0.02)	3.90 (0.01), H6	2-1
	1.23 (0.44)		9.12 (0.02)	9.34 (0.01), H2'	2-1
	1.64 (0.15)		8.50 (0.01)	9.14 (0.006), H4'	2-1
	1.23 (0.21)		7.67 (0.03)	8.26 (0.01), H5'	2-1
	1.37[b]				
1.82 (0.25)	1.03 (0.21)	1.21 (0.06)			2-3
15-16	4.8-5.0	0.9-1.1			2-4[c]

Note: Values given in parentheses are one standard deviation.
[a]Equation used to calculate constants.
[b]Average of K_a values found using eq 2-1.
[c]Using absorbance changes at 238 nm, 25 °C, and 0.15 M ionic strength in H_2O.

Table 2-3. Observed Chemical Shifts for H2', H4', and H5' of the Amino Ketone Form of Anabaseine in D_2O at 22 °C and 0.6 M Ionic Strength.

pD	Chemical Shifts / ppm[a] H2'	H4'	H5'
4.47	9.177	8.610	7.810
4.37	9.171	8.623	7.818
4.11	9.199	8.705	7.889
3.64	9.275	overlap	8.062
3.0	9.312	9.044	8.183
2.2	9.342	9.120	8.248

[a]TSP was used as an internal standard.

isotope effect (0.50) is not unlike that for 3-acetylpyridine (pK_a 3.2, H_2O [31]), a reasonable model.

The pD-hydrolysis profile and the hydrolysis equilibrium constant K_H

That the system is rapidly reversible and hence at equilibrium was demonstrated by taking a reaction mixture, changing its pD and redetermining the substrate ratio. Thus, following observation of the hydrolysis ratio of a sample having pD 4.37, the pD was increased to 9.22 by the addition of carbonate ion and the new ratio of open-chain to cyclic substrate was determined two hours later. All the substrate now existed as cyclic imine as was found for a fresh sample of similar pD made directly from anabaseine. Similarly, a change was made in the opposite direction; a spectrum was recorded immediately after an alkaline sample was acidified

from pD 8.20 to 4.82. Again the ratio was the same as that obtained on a fresh sample of similar pD.

The hydrolysis equilibrium is defined by the reaction 2-2 + D_2O → 2-3 where $K_H = [2\text{-}3]/[2\text{-}2]$. However, NMR area measurements provide the total amounts of each of the two components, i.e., (2-3 + 2-4) and (2-1 + 2-2) and not just 2-3 and 2-2 alone. Therefore, the sum must be corrected for the amount of the unwanted component to obtain the value for K_H. This is accomplished easily by eq 2-2 with the aid of the appropriate K_a values along with the concentration of acid obtained from pD measurements. The fraction of amino ketone where the pyridine is unprotonated is given by $K_{a2}/([D^+]+K_{a2})$; the fraction of imine where the imine nitrogen is protonated is given by $[D^+]/([D^+]+K_{a1})$.

$$K_H = \frac{[\text{open-chain}]_{tot}}{[\text{ring-closed}]_{tot}} \cdot \frac{K_{a2}/([D^+] + K_{a2})}{[D^+]/([D^+] + K_{a1})} \qquad (2\text{-}2)$$

Prior to computer fitting, eq 2-2 was rewritten to express the fractional amount of total substrate present as the cyclic imine (free base 2-1 and its conjugate acid 2-2) rather than as the ratio of [2-3]/[2-2] (eq 2-3).

$$\text{fraction of cyclic imine} = \frac{K_{a2}(K_{a1} + [D^+])}{K_{a2}(K_{a1} + [D^+]) + K_H[D^+](K_{a2} + [D^+])} \qquad (2\text{-}3)$$

The latter fraction (eq 2-3) ranges in value from 0 to 1 whereas the other tends to a very large value at low pD owing

to the small amount of 2-2 thereby causing computational problems. Equation 2-3 was then solved with the aid of a microcomputer program using nonlinear regression analysis. From the data in Figure 2-4 it was possible to obtain the three relevant equilibrium constants K_{a1}, K_{a2} and K_H to describe quantitatively the changes in the concentrations of the four species 2-1 - 2-4 as a function of pD. Table 2-2 lists the calculated values and Figure 2-4 shows the calculated curve through the data points.

Worthwhile is a comparison of the K_a values obtained by using the variation in chemical shifts, eq 2-1, and by the overall fitting technique based on eqs 2-2 and 2-3. There is satisfactory agreement, Table 2-2. The values for K_{a1} overlap within one standard deviation for the two approaches while the value for K_{a2} derived from a consideration of all the data in Figure 2-4 is 25% smaller than the average found by the chemical shift method, reasonable agreement for NMR analysis. Our results are self-consistent.

The value of 1.21 for K_H shows that almost equal amounts of the two monocationic species 2-2 and 2-3 are present with the open-chain ketone being favored slightly.

Ultraviolet Spectra of Aqueous Anabaseine Solutions

Another approach using ultraviolet absorption spectra was taken to examine the hydrolysis reaction. Spectral changes were used to follow the reaction, this time in H_2O at

25 °C and at a lower salt concentration, 0.15 M. Again spectra were taken as a function of solution acidity and 238 nm was selected as the analytical wave length because of the large and complex absorbance changes. Figure 2-5 shows how the absorption varies with acidity over the pH interval 1 to 12.

Attempts to obtain equilibrium constants were more difficult and unsatisfactory because unlike the NMR method individual species could not be observed. Our nonlinear regression program was employed to estimate constants from eq 2-4 where [2-1 - 2-4] denotes the total amount of substrate, Figure 2-1, ε represents the molar absorptivity, and F indicates the fraction of the total amount of substrate present in solution as one of the four forms.

$$ABS_{obs} = (\varepsilon_{2-1}F_{2-1} + \varepsilon_{2-2}F_{2-2} + \varepsilon_{2-3}F_{2-3} + \varepsilon_{2-4}F_{2-4}) \cdot [\underline{2\text{-}1} - \underline{2\text{-}4}] \qquad (2\text{-}4)$$

$$F_1 = \frac{K_{a1}K_{a2}}{K_{a1}K_{a2} + K_{a2}[H^+] + K_H K_{a2}[H^+] + K_H[H^+]^2} \qquad (2\text{-}5)$$

Equation 2-5 serves as an example of how this fraction may be expressed in terms of the appropriate equilibrium constants and the $[H^+]$. The fractional amount of the total substrate present as 2-1 is indicated. The other fractions may be written by taking the same denominator and replacing the

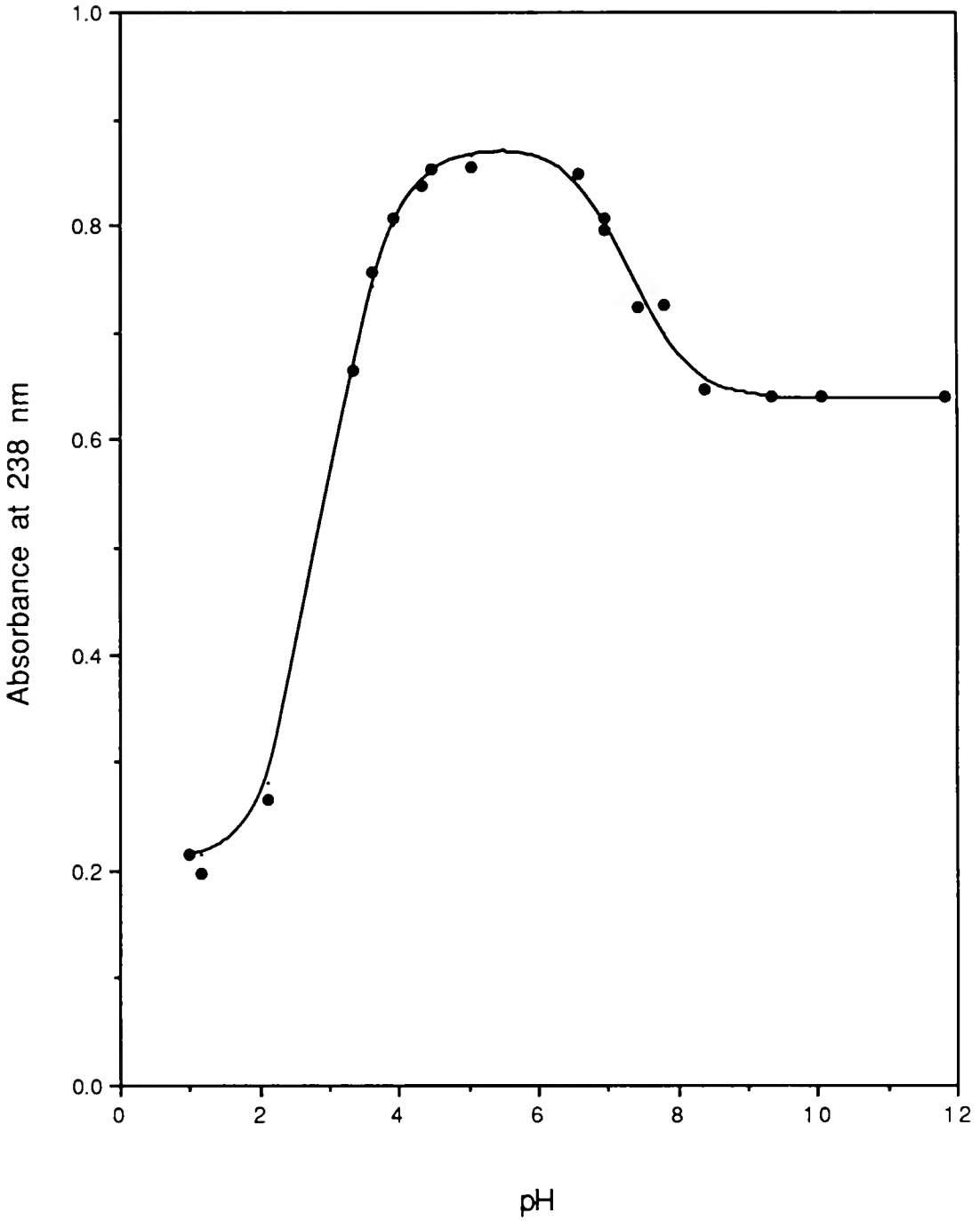


Figure 2-5. A titration curve for anabaseine according to Figure 2-1 based on ultraviolet absorption data collected at 238 nm and 25 °C using H_2O at 0.15 M ionic strength. The filled circles are experimental absorbances and the curve was calculated using eq 2-4 and 2-5 and the constants in Table 2-2.

numerator with one of the three remaining terms in the denominator. Thus, the second term when placed in the numerator gives F_{2-2}, the third F_{2-3}, and the fourth F_{2-4}. Additional unknowns, the molar absorptivities or ε values, are not present in the NMR equation but are found in eq 2-4. Estimates of these were made as follows. The ε value at high pH (10-12) was assumed to be due to free imine, 2-1, and that at low pH (1.1) dication, 2-4. The NMR ratios near neutrality where both 2-2 and 2-3 exist almost exclusively were considered in an attempt to dissect the observed absorbance and obtain estimated ε values for 2-2 and 2-3. A unique fit to eq 2-4 was not found because of the uncertainty in these values. For example, when the initial estimates for 2-2 and 2-3 were varied by about a factor of two from those reported in the Experimental Section, the value of K_H had a very large uncertainty and so these estimates were rejected. The final values seem to be reasonable. The points in Figure 2-4 are the experimental values and the line is that calculated by the equilibrium constants given in Table 2-2, the molar absorptivities in the Experimental Section and eq 2-4, showing a satisfactory fit.

Discussion

Anabaseine Equilibria

The rapidly equilibrating set of four components, Figure 2-1, resulting from the hydrolysis of anabaseine is described quantitatively for the first time. The titration curve, Figure 2-4, obtained for this complex mixture may be expressed in another way, Figure 2-6, that shows how the concentrations of each one of the four components varies with solution acidity. Figure 2-6 was constructed using $[D^+]$ and fractions defined by eq 2-5 and its counterparts where the numerator for each fraction changes as explained in the Results. Open-chain dication 2-4 predominates at low pD and cyclic free base 2-1 is the major component at high pD. Over a wide acidity range near neutrality and under physiological conditions the two monocations 2-2 and 2-3 are essentially the only two materials present in solution, acyclic ketone slightly predominating. At pD 7 for example, there is 8% of 2-1, 42% of 2-2 and 50% of 2-3. This composition will be different in H_2O. Making the reasonable assumptions that K_H will not change significantly but that the two relevant pK_a values will decrease by 0.5 due to the solvent isotope effect gives rise to a new equilibrium distribution: 2-1, 21%, 2-2, 36% and 2-3, 43%. There is a major increase in the amount of 2-1 on going to H_2O.

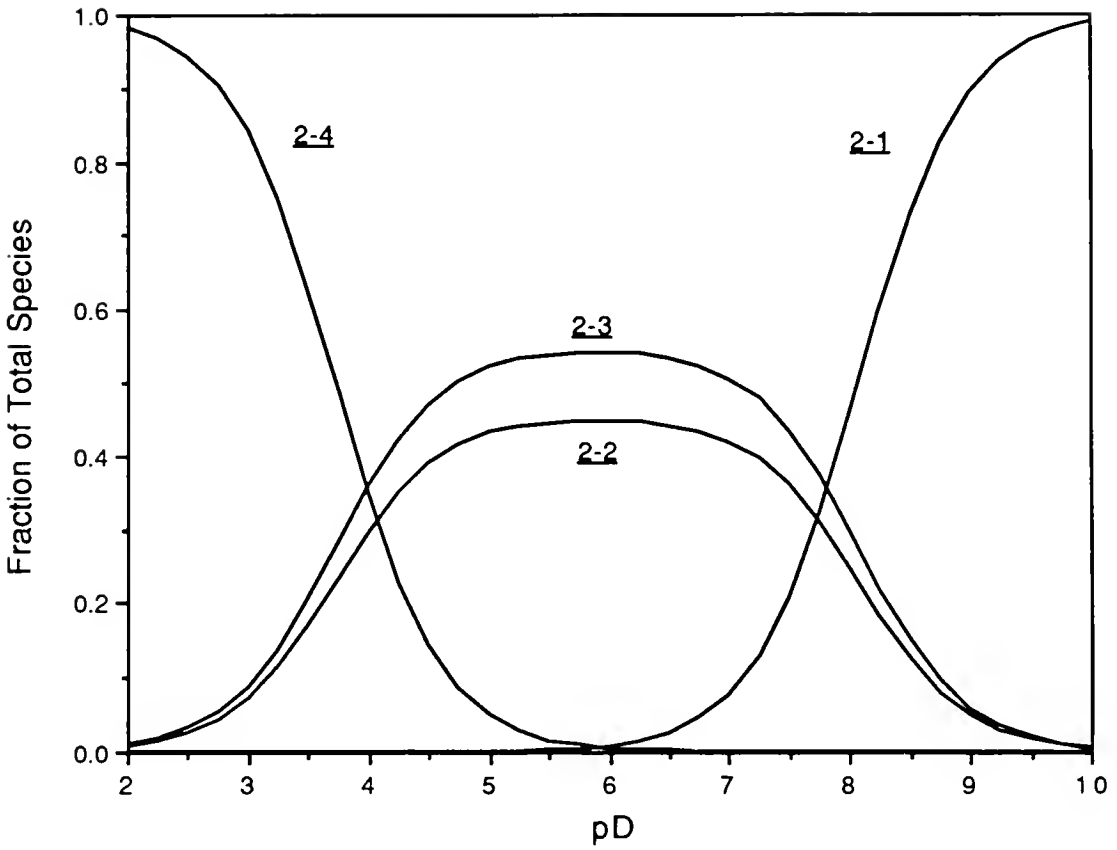
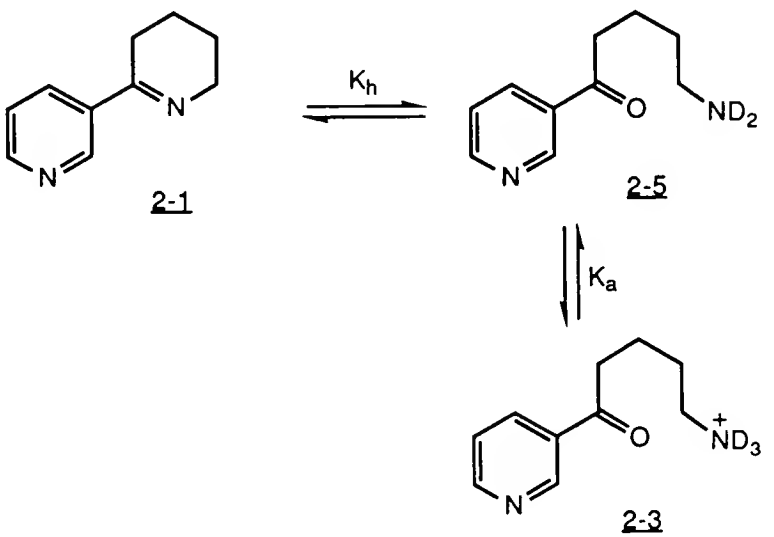


Figure 2-6. A plot showing how each of the four components of the anabaseine equilibrium given in Figure 2-1 varies with pD.

The K_H term describing the hydrolysis equilibrium in terms of the two monocationic constituents may be written in an alternate form, as an equilibrium between the cyclic free base 2-1 and the open-chain free base 2-5, where 2-5 is the conjugate base of acid 2-3 (eq 2-6). This new constant is given by the expression $K_h = (K_H \times K_{a3})/K_{a1}$ where K_{a3} equals $[D^+]\ [2\text{-}5]/[2\text{-}3]$. Using 10.5 as an estimate of pK_{a3} (a reasonable estimate based on calculated values for similar amines in Chapter 3), the value for the alkyl ammonium ion, provides a value of 3×10^{-3} for K_h, indicating that only 0.3%

of 2-5 exists in the presence of 2-1 at high pD. Although K_h expresses the hydrolytic equilibrium in terms of neutral rather than charged substrates, we prefer K_H. As Figure 2-6 shows, K_H pertains to the predominant constituents of the equilibrium under most acidity conditions whereas K_h does not.



(2-6)

The pK_a values derived from samples in D_2O and in H_2O, Table 2-2, differ by 0.5 to 1. Neglecting the small variation in temperature between our two studies, pK_a values for pyridinium ions in heavy water are expected to be some 0.4 to 0.5 higher [32, 33], reflecting the greater acidity of D_3O^+ over H_3O^+. The difference in the ionic strength (0.15 vs 0.6 M) also leads to a small increase in pK_a value. A large displacement in the position of the titration curve along the axis expressing solution acidity for a related substance, myosmine, which contains a 1-pyrroline in place of the

1-piperideine ring of anabaseine, has also been reported when the solvent was changed from heavy to light water. The hydrolysis equilibrium for myosmine is considered in Chapter 3. The main result of the spectrophotometric analysis is confirmation of the value of K_H.

Apparent pK_a Value

A pK_a value of 6.7 already has been reported for anabaseine based on a titration with aqueous HCl in H_2O containing 5% methylcellosolve at 25 ^oC [34]. Our work shows that this value cannot be for the pure substance but rather for the mixture given in Figure 2-1. That is, during the titration hydrolysis of 2-1 occurs and this produces the more basic open-chain, aliphatic amine 2-5. The K_a value is skewed to include the more basic substance that will be present as its conjugate acid 2-3. The reported dissociation constant is an apparent K_a value (K_{app}) given by $K_{app} = [H^+][2\text{-}1]/([2\text{-}2] + [2\text{-}3])$ and not K_{a1} as desired.[2] This apparent dissociation constant is related to the true dissociation constant K_{a1} as follows: $K_{app} = K_{a1}/(1 + K_H)$ which on a logarithmic scale is $pK_{app} = pK_{a1} + 0.34$, including the

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[2] Careful consideration of the relevant equilibria lead to a more complex equation. At high pD, $K_{app} = [D^+]([2\text{-}1] + [2\text{-}5])/([2\text{-}2] + [2\text{-}3])$ that may be transformed to $K_{app} = K_{a1}(1/(1+K_H)) + K_{a3}(K_H/(1+K_H))$. The latter equation has the same form as that of NMR eq 2-1: in both cases the observed value is a population weighted average of two constants, in one case the limiting chemical shifts and in the other the K_a values. Because the amount of 2-5 is so small under our conditions, the second term of the K_{app} equation may confidently be neglected.

logarithmic value for $(1 + K_H)$. In this case the correction for the presence of the amine component is not very large because K_H is not very large. Moreover, this analysis is supported by our titration curve, Figure 2-4, where careful examination of the inflection point at high pD shows that this point does not have the value for pK_{a1} of 7.8 but rather for pK_{app} of 8.1 as expected. Correcting our pK_a value of 7.8 for solvent isotope and salt effects by as much as 0.7 gives a value of 7.1 for H_2O. This is only in fair agreement with the corrected value of 6.4 that previous workers obtained by titration.

While our assumption concerning the absence of protonation of the pyridine ring of the cyclic imine is not strictly correct, little error should be introduced into our value of the K_a for the pyridine ring of the ketone because the degree of protonation is likely to be small. Inclusion of diprotonated imine, 2-6, would give an apparent $pK_{app} = [D^+]([2\text{-}2]+[2\text{-}3])/([2\text{-}4]+[2\text{-}6])$. If [2-6] is small relative to [2-4], it can be neglected to give $K_{app} = [D^+]([2\text{-}2]+[2\text{-}3])/[2\text{-}4]$ which is equal to $K_{app} = K_{a2}(1+K_H)/K_H$. Converting to a logarithmic scale gives $pK_{app} = pK_{a2} + 0.26$. Substituting in our calculated value of 3.99 for pK_{a2} gives $pK_{app} = 3.7$ which nicely reproduces the inflection point on our titration curve at low pD, Figure 2-4, showing that the contribution from 2-6 can confidently be neglected. Moreover, our data at very low pD are sparce and attempts to include the additional K_a value

into the computer fit had no effect on the values for the other constants.

Conclusions

The composition of aqueous solutions of 2-1 is described quantitatively for the first time. The state of protonation as well as the degree of conversion to the open-chain constituents is presented in Figures 2-4 and 2-6 as a function of the acidity of aqueous solutions. These findings make it possible to develop a strategy to ascertain the active form responsible for the pharmacological action of 2-1.

CHAPTER 3
COMPUTER SIMULATION OF pH-DEPENDENT RING-CHAIN EQUILIBRIA FOR OTHER COMPOUNDS OF BIOLOGICAL INTEREST

Introduction

Encouraged by our success with anabaseine, pH-dependent ring-chain equilibria for other biologically significant molecules were simulated using the appropriate models with the iterative nonlinear regression program. Other compounds studied include the tobacco alkaloid myosmine (3-1)[1] [8, 35] which contains a 5-memebered 1-pyrroline ring instead of the 1-piperideine ring of anabaseine, its cationic N-methylated derivative 3-2, and a derivative containing an N-methylated pyrrolinium ring and a phenyl ring in place of the 3-pyridyl ring 3-3. In aqueous solutions, myosmine and its derivatives are also hydrolyzed to produce mixtures of cyclic imine and open-chain amino ketone forms. The pD-hydrolysis profiles for these compounds have been reported in the literature and are constructed based on ^{1}H NMR data [8, 9]. No attempt was made previously to describe quantitatively these titration curves and calculate equilibrium constants. These data were used along with the nonlinear regression program and models

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[1] The hydrolysis product of myosmine is named poikiline [35]. Myosmine is also known as 3-(3,4-dihydro-2H-pyrrol-5-yl)-pyridine, 3-(1-pyrrolin-2-yl)pyridine, and as 2-(3-pyridyl)-1-pyrroline.

for the hydrolysis equilibria to obtain K_a and K_H values for the species involved [4].

A fourth compound studied was the oxidation product of spermine and spermidine. Spermine[2] and spermidine[3] are two of several aliphatic polyamines important in the control of proliferative processes [36]. They may be oxidatively degraded either chemically or enzymatically [37]. The structure of the product from the enzymatic reaction depends on both the nature of the polyamine and the enzyme [38], in some instances being the same as that from the nonenzymatic reaction [37]. One of these products has a structure that has been controversial. It formally arises from the cyclization of a diamino aldehyde. The early assignment of a monocyclic 2-pyrroline or enamine structure 3-5 provides a compound with properties that are not entirely consistent with some of those reported for the oxidation product and so 3-6 was proposed [39]. The latter is a racemic, bicyclic aminal having been formed by the spontaneous cyclization of the same diamino aldehyde precursor.

More recently, a family of rapidly interconverting structures including 3-6, 3-7 and 3-8 has been suggested for the oxidized material based on a careful consideration of ^1H and ^{13}C NMR spectra of a synthetic sample in a series of aqueous solutions of varying acidity (Figure 3-7) [10]. Bicyclic ion 3-7 consists of two rapidly interconverting

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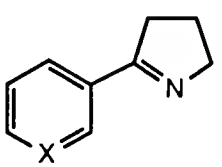
[2] N,N'-Bis(3-aminopropyl)-1,4-butanediamine.
[3] N-(3-Aminopropyl)-1,4-butanediamine.

conjugate acids of secondary 3-7a and tertiary 3-7b amines. Its monocyclic conjugate acid 3-8 is a 3-ammoniopropyl-1-pyrrolinium dication. This system includes a tautomeric ring-chain equilibrium between 3-7a and 3-9 rather than a hydrolytic equilibrium as in the previous systems. The pH-dependent ring-chain equilibrium was simulated using the model in Figure 3-7 and the published NMR data to obtain equilibrium constants [11].

Results

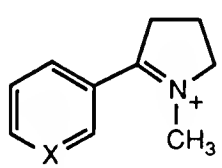
pD-Hydrolysis Profiles for Myosmine and Analogs

Figures 3-1, 3-2, and 3-3 show how the composition of solutions 3-1, 3-2, and 3-3, respectively, in D_2O change with pD. Again, the points in the figures are experimental values, estimated from the published figures and the curves are those calculated from our computer simulations.



3-1, X = N

3-4, X = CH



3-2, X = N

3-3, X = CH

The published data for 3-1 [8], 3-2 [9] and 3-3 [9] had not been subjected to a computer analysis and so equilibrium

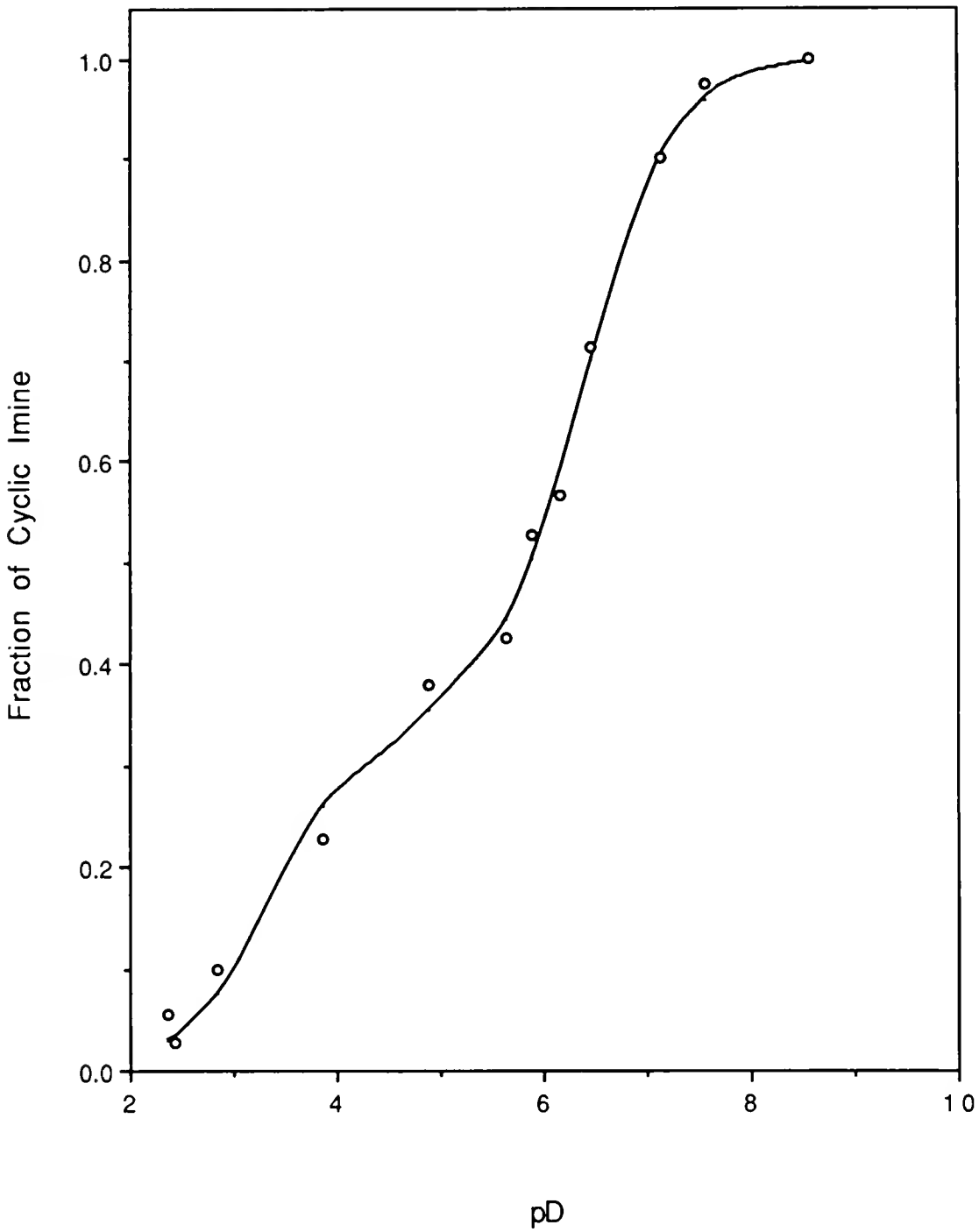


Figure 3-1. A titration curve for the hydrolysis of myosmine (3-1) in D_2O. The open circles are experimental values representing the fraction of imine and its conjugate acid taken from ref 8. The curve was calculated based on eq 2-3 and the equilibrium constants obtained from the computer fit.

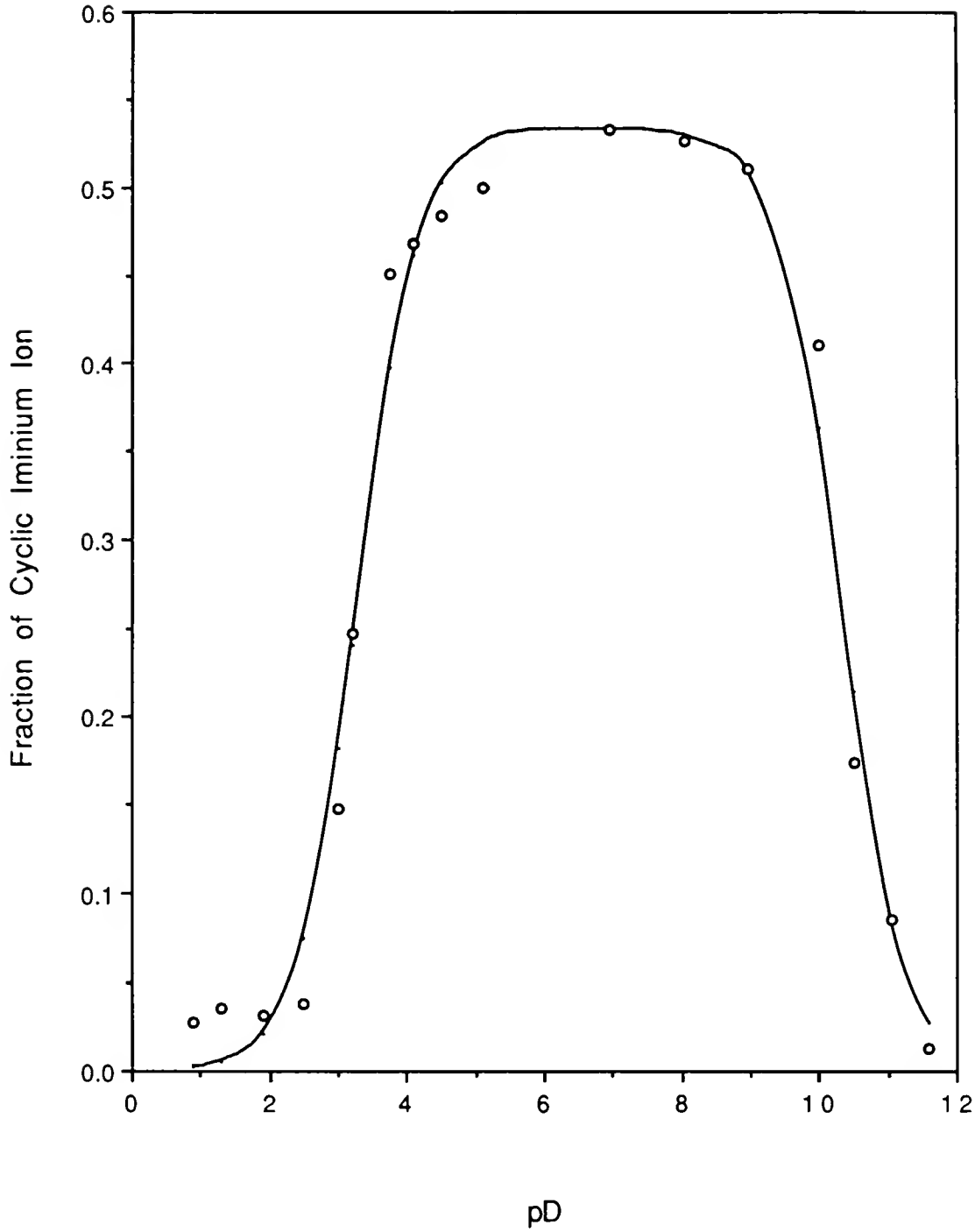


Figure 3-2. A titration curve for the hydrolysis of N-methylmyosmine (3-2) in D_2O. The open circles are experimental values representing the fraction of iminium ion taken from ref 9. The curve was calculated based on eq 3-1 and the equilibrium constants obtained from the computer fit.

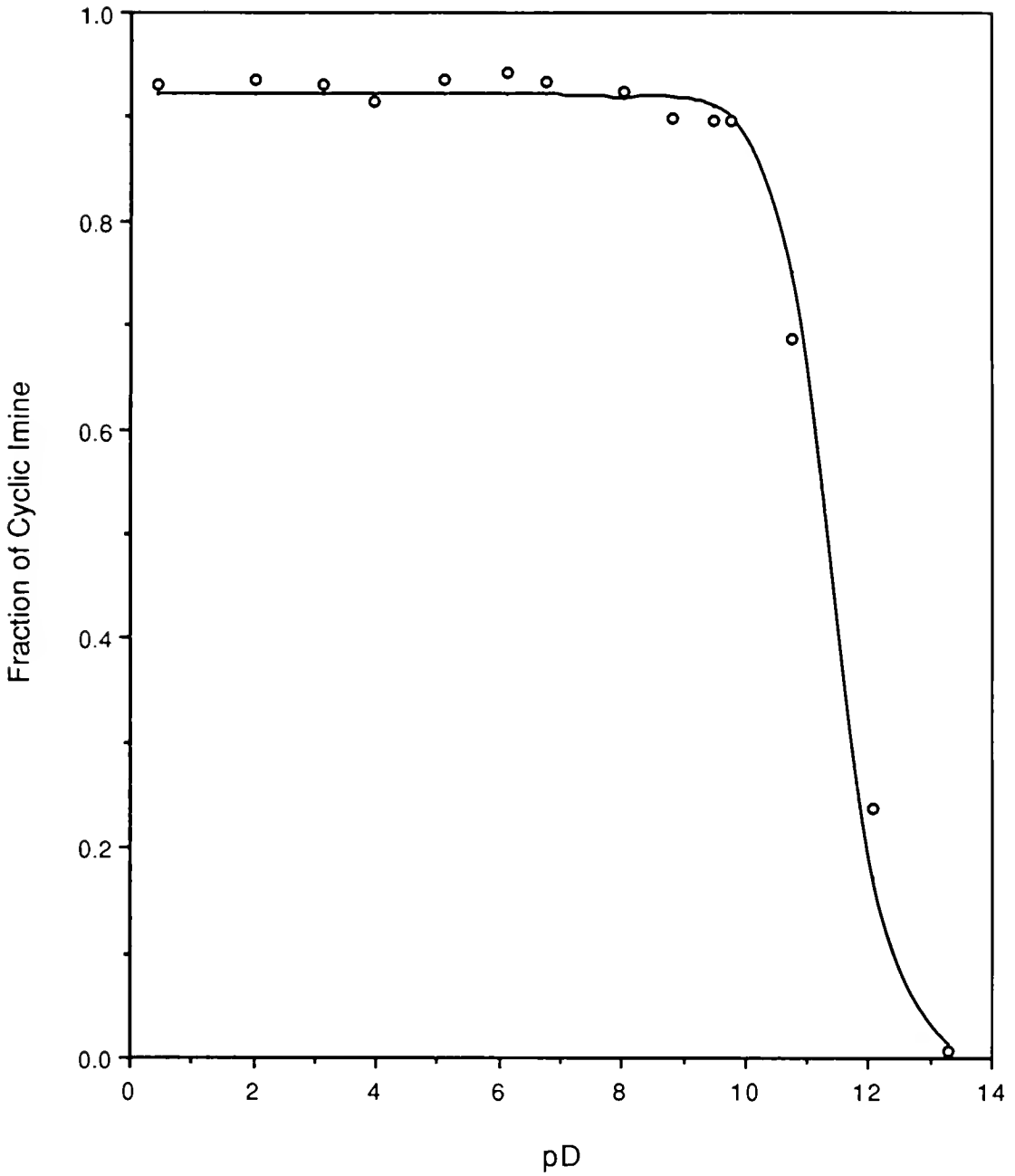


Figure 3-3. A titration curve for the hydrolysis of 1-methyl-2-phenyl-1-pyrrolinium ion (3-3) in D_2O. The open circles are experimental values representing the fraction of iminium ion taken from ref 9. The curve was calculated based on eq 3-2 and the equilibrium constants obtained from the computer fit.

constants describing the titration curves had not been defined. Our analysis allows these values to be extracted from the data for the first time.

Myosmine

The pD-hydrolysis profile for myosmine was simulated using the same equation (eq 2-3) and model (Figure 2-1) as those used for anabaseine. The calculated equilibrium constants appear in Table 3-1 and the curve through the data points in Figure 3-1 was calculated using eq 2-3 and the constants.

1-Methyl-2-(3-pyridyl)-1-pyrrolinium ion

The hydrolysis profile for 1-methyl-2-(3-pyridyl)-1-pyrrolinium ion (3-2) is different in shape from those for 2-1 and 3-1 in that at high pD values the uncharged open-chain amino ketone predominates rather than neutral, cyclic compound as in the cases of 2-1 and 3-1. However, with 3-2 the cyclic form is constrained to be a cation due to quaternization instead of protonation of the nitrogen atom and so the more reactive cyclic iminium ion hydrolyzes to the amino ketone at high pD. Because of the presence of large amounts of this open-chain material at high pD, the scheme in Figure 2-1 does not correctly describe the hydrolysis of 3-2. K_{a1} is not relevant and K_{a3} must be added to reflect conversion of the open-chain alkylammonium ion to its conjugate base (Figure 3-4). Again, K_H is defined as the ratio of the ketoammonium ion to the cyclic iminium ion.

Table 3-1. Equilibrium Constants Obtained from Analysis of Literature Data for the Hydrolysis of Cyclic Imines.

Compound	K_{a1}	K_{a2}	K_{a3}	K_H	eq[a]	ref[b]
3-1	1.20×10^{-6}	2.66×10^{-4}		1.89	2-3	[8]
3-2		2.42×10^{-4}	1.00×10^{-10}	0.88	3-1	[9]
3-3			4.95×10^{-11}	0.085	3-2	[9]
3-4				0.18	--	[8]

[a] Equation used to calculate equilibrium constants.
[b] Reference which contains NMR data for pD-hydrolysis profiles.

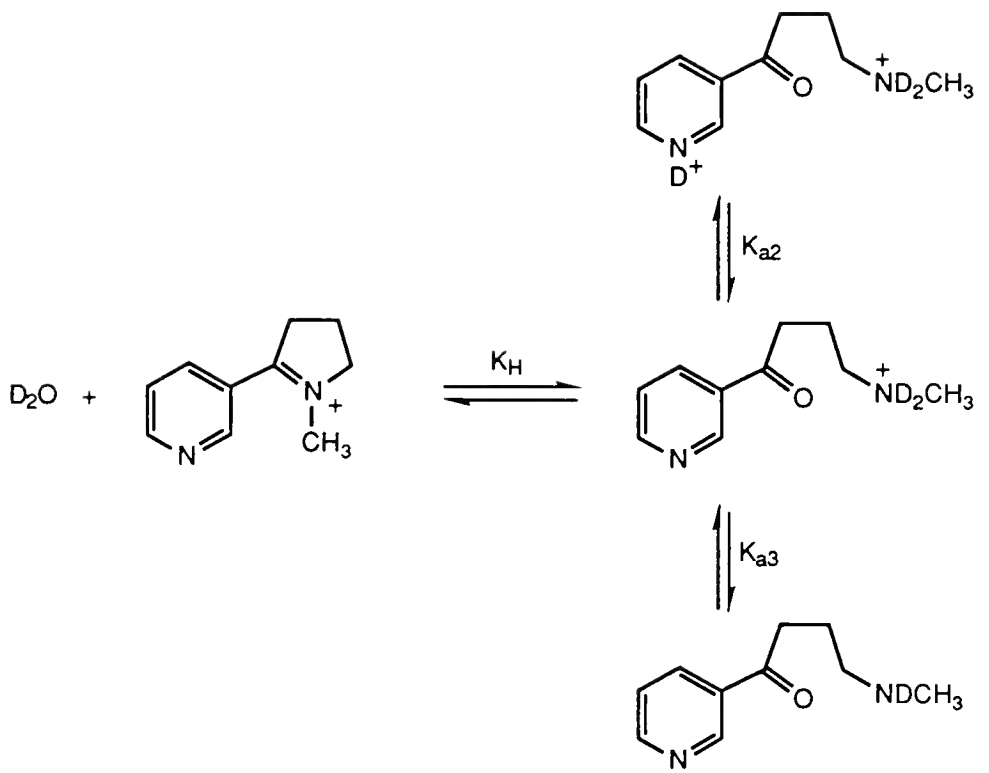


Figure 3-4. Model for N-methylmyosmine (3-2) equilibria in aqueous solution.

Hence, eq 2-2 which was used for anabaseine and myosmine must be modified; the concentration ratio of open-chain to cyclic substrate must now be multiplied by the fraction $[D^+]K_{a2}$ / $([D^+]^2 + [D^+]K_{a2} + K_{a2}K_{a3})$ instead of the fraction given therein to reflect the amount of open-chain material present as the monocation. Again, the equation was rearranged and written in terms of fraction of free imine to avoid computational problems (eq 3-1). This consideration produces a satisfactory fit (Figure 3-2).

$$\text{Fraction of Iminium Ion} = \frac{K_{a2}[D^+]}{K_{a2}[D^+] + K_H([D^+]^2 + K_{a2}[D^+] + K_{a2}K_{a3})} \qquad (3\text{-}1)$$

The hydrolysis profile for 3-2 could be made even more complex because the cyclic iminium ion is the conjugate acid of an enamine and could be deprotonated to the enamine at high pD values [40]. However, after a careful search the enamine was not detected [9] and so it was not included in our scheme. Redefining K_{a3} as a dissociation constant to reflect the formation of enamine conjugate base with the same value for K_{a3} rather than to yield acyclic amine base would have no influence on the shape of the titration curve.

In the most acidic solutions for 3-2 there are some deviating points (Figure 3-2). It is not clear whether these points reflect the incursion of a new conjugate acid of substrate or simply represent NMR errors in estimating small amounts of minor component.

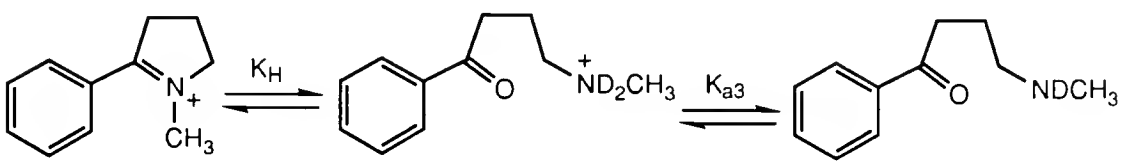
1-Methyl-2-phenyl-1-pyrrolinium ion and 2-phenyl-1-pyrroline

Two other reports concerning the equilibrium hydrolysis of 1-pyrrolines are of interest because they provide additional insight into the influence of structure on the value of K_H. 1-Methyl-2-phenyl-1-pyrrolinium ion (3-3) and 2-phenyl-1-pyrroline (3-4) have a phenyl substituent in place of the pyridyl ring of myosmine, the former also an N-methyl group. The titration curve for 3-3 resembles that for 3-2 at high pD in that open-chain material is favored while acidic solutions show no variation in composition because of the lack of a second basic group, the pyridine nitrogen (Figure 3-3) [9]. Again, we were able to fit the reported titration

curve for 3-3 based on the model in Figure 3-5 and using eq 3-2 with the computer program, equilibrium constants are listed in Table 3-1.

$$\text{Fraction of Iminium Ion} = \frac{[D^+]}{[D^+] + K_H([D^+] + K_{a3})} \qquad (3\text{-}2)$$

Although an enamine equilibrium could be incorporated into the hydrolysis model, we find this to be unnecessary. Even if the situation is made more complex by the inclusion of a pK_a value for an enamine (12.7), both pK_{a3} and K_H are essentially unchanged, now being 10.2 and 0.083, respectively.



3-3

Figure 3-5. Model for equilibria of 1-methyl-2-phenyl-1-pyrrolinium ion (3-3) in aqueous solution.

The titration curve was not presented for 3-4 but from the reported equilibrium composition [8] we were able to estimate a value for K_H, Table 3-1.

Oxidation Product of Spermine and Spermidine

A titration curve based on proton chemical shifts of a synthetic sample of the oxidation product dissolved in D_2O was reported and the data points are reproduced in Figure 3-6. According to this report [10] bicyclic 3-6 converts to monocyclic 3-8 on acidification. The first analysis starts with the pair of equilibrating bicyclic monodeuteronated cations 3-7a and 3-7b, said to be the major forms in neutral water (Figure 3-7) [10]. They are in equilibrium with monocyclic, tautomeric monocation 3-9. This amine, expected to be more basic than the aminal because it resembles a simple aliphatic amine with a remotely situated electron-withdrawing group, accepts the deuteron, trapping the substrate in its monocyclic form to give the observed product 3-8. According to this model the fraction (F) of the total amount of substrate present as imine is given by eq 3-3 which assumes that in the acidity interval in question there are only three significant forms present, 3-7, 3-8 and 3-9 [41]. K_{a1} is the dissociation constant for 3-8 going to 3-9 while K_T denotes the ratio of ring-chain tautomers [42] [3-7a]/[3-9] and K_t gives the ratio of acids [3-7b]/[3-7a]. Because K_{a1} is small relative to the concentration of acid $[D^+]$ in eq 3-3 it may be neglected as indicated in eq 3-4 where the term $K_{a1}K_T(1+K_t)$ is a constant equal to K_{app}.

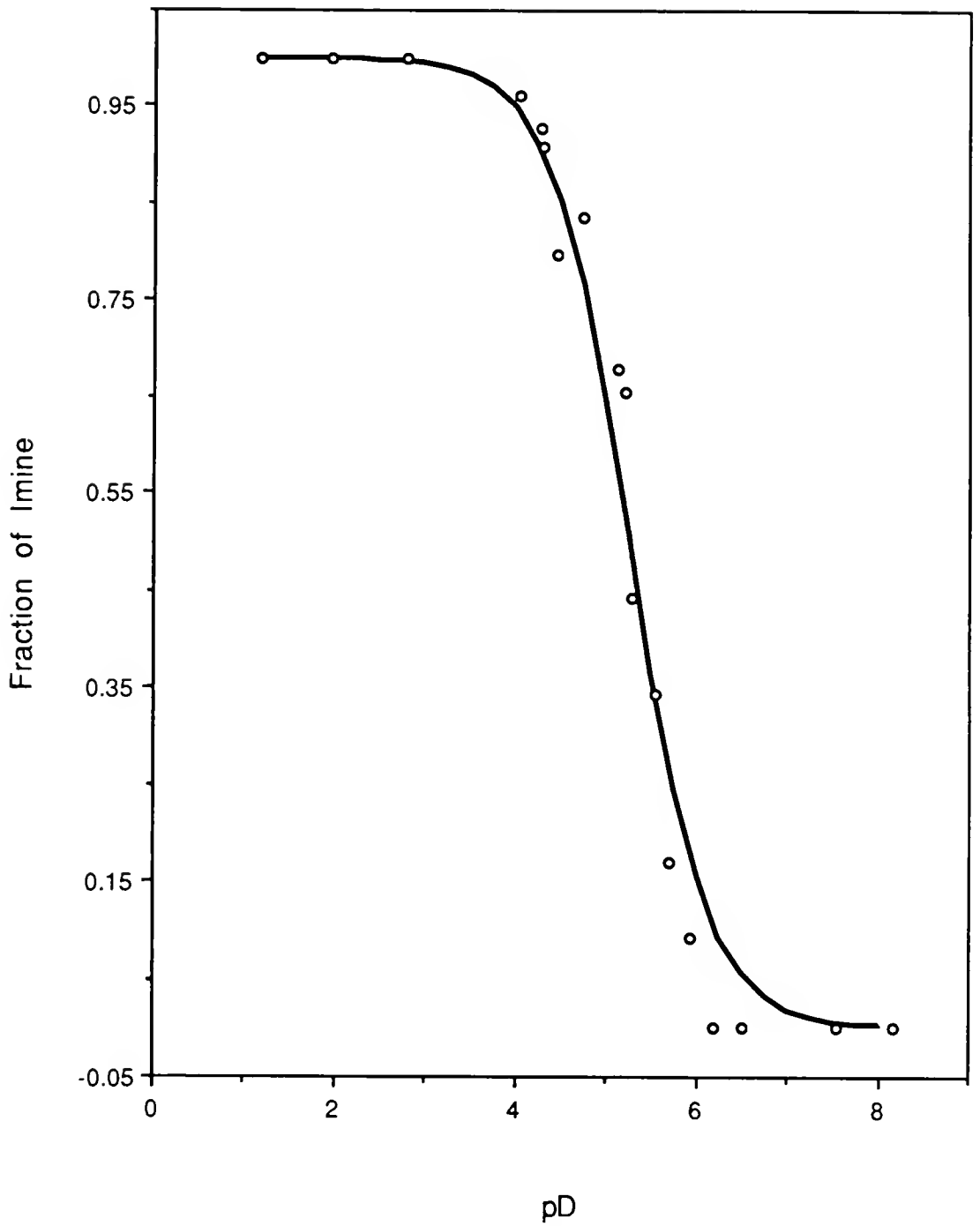


Figure 3-6. Titration curve for the oxidation product of spermine and spermidine in D_2O. The open circles are experimental values taken from ref 10 and the solid line was calculated using eq 3-4 and $K_{app} = 5.47 \times 10^{-6}$.

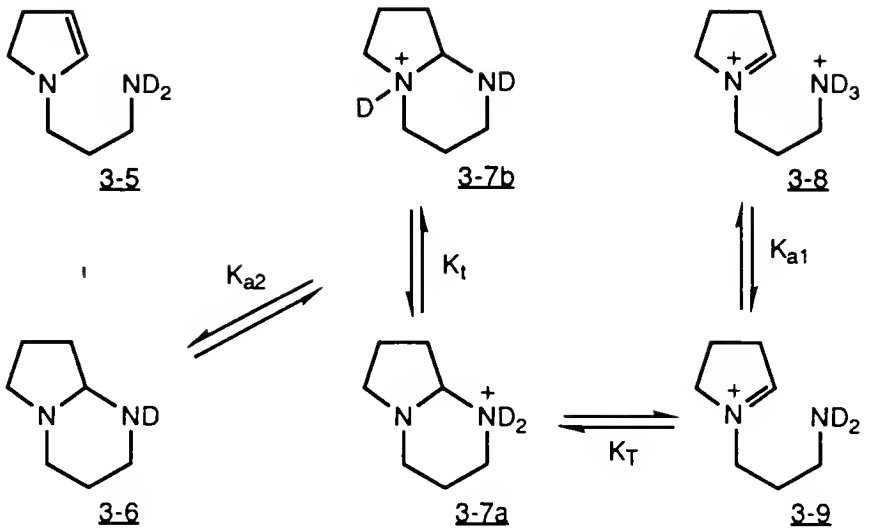


Figure 3-7. Model the equilibria associated with aqueous solutions of the oxidation product of spermine and spermidine.

K_{app} is the apparent K_a value as given by the half-titration point.

$$F = \frac{[3\text{-}8] + [3\text{-}9]}{[3\text{-}7] + [3\text{-}8] + [3\text{-}9]} = \frac{[D^+] + K_{a1}}{[D^+] + K_{a1} + K_{a1}K_T(1 + K_t)} \qquad (3\text{-}3)$$

$$F \approx \frac{[D^+]}{[D^+] + K_{app}} \qquad (3\text{-}4)$$

The experimental data as taken from the reported titration curve [10] have been fit using a nonlinear regression microcomputer program and eq 3-4. The fit shown by the solid line in Figure 3-6 is satisfactory except that our calculated values for the fraction of imine appear to be slightly too large at a pD of about 6. If this analysis is accepted, then the K_{app} value of 5.47×10^{-6} (pK_{app} 5.26) with a standard deviation of 0.56×10^{-6} may be converted to its two

associated constants using an estimate of 9.68 for pK_{a1} [43] from a model, the dideuteronated form of 1,3-diaminopropane.[4] The value for $K_T(1+K_t)$ becomes 2.6×10^4. Since tertiary alkylammonium ions are likely to be slightly stronger acids than their corresponding secondary relatives the term $(1+K_t)$ is expected to be about 1 in value and so K_T is approximately 2.6×10^4 showing that only a very small amount of the open-chain amino tautomer is present along with the deuteronated cyclic aminal.

The titration curve can also be simulated using a more complex expression starting with aminal 3-6 as its free base. In this simulation two hydrons are involved in the overall conversion to monocyclic dication 3-8. As shown in Figure 3-8 the fit in the pD 6 region is much better. According to this model the fraction (F) of total substrate present as the monocyclic imine 3-8 and 3-9 is given by eq 3-5 where the previously employed K values are as represented earlier and K_{a2} stands for the combined dissociation constant for the mixture of 3-7a and 3-7b going to 3-6 (Figure 3-7). Again, since K_{a1} is small relative to $[D^+]$ over the pD range of the titration, the term $[D^+]K_{a1}$ can be neglected and the constants combined into two where K_{1app} equals $K_{a1}(K_T(1+K_t))$ and K_{2app} equals $K_{a2}/(1+K_t)$, eq 3-6.

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[4] The reported pK_a of 8.88 at 25 °C (H_2O) is statistically corrected for two equivalent acidic sites (0.30) and a solvent isotope effect of 0.50[44] to give the value of 9.68.

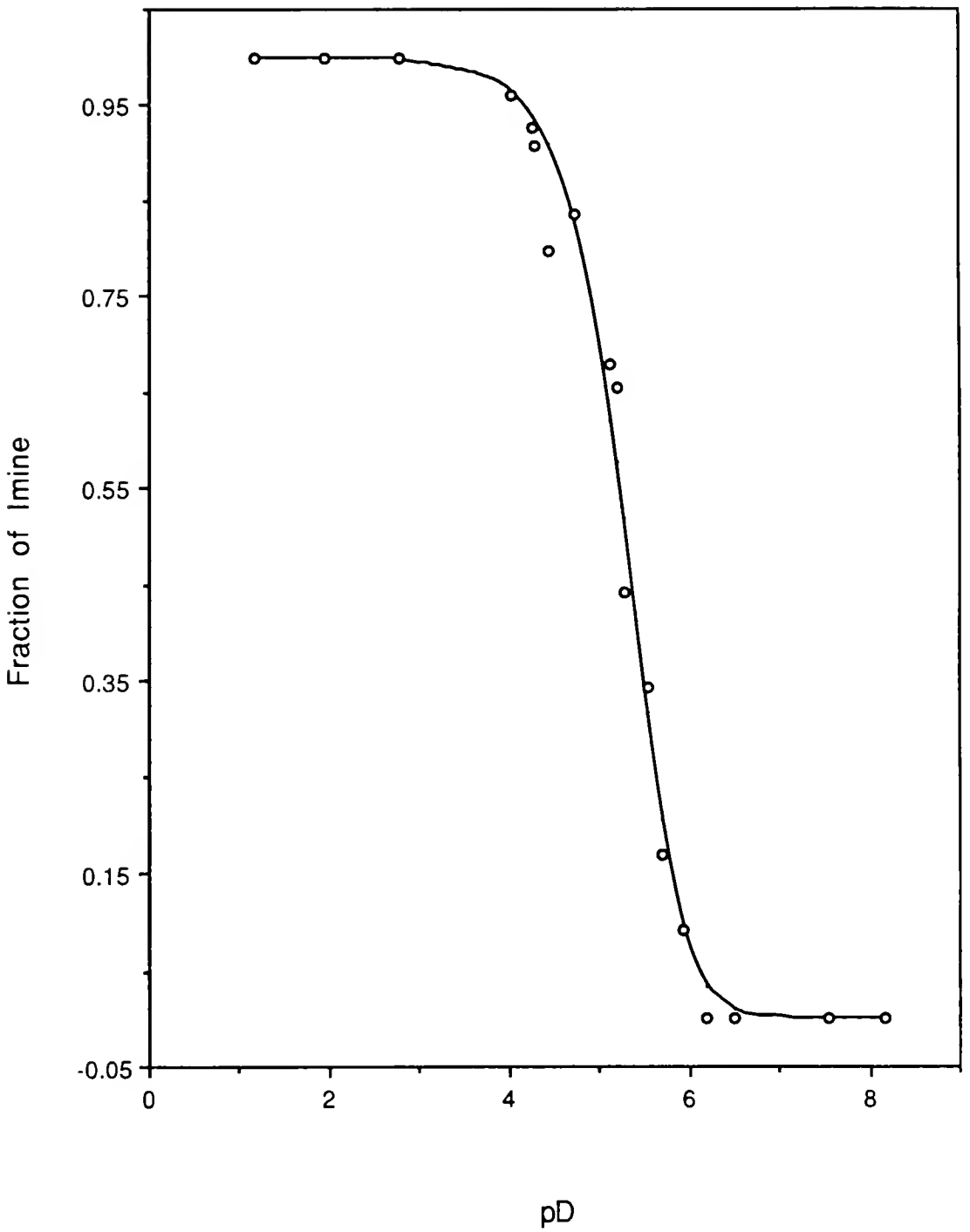

Figure 3-8. The same titration data as in Figure 3-6 but this time the curve is calculated using eq 3-6 with K_{1app} = 3.33 x 10^{-6} and K_{2app} = 2.67 x 10^{-6}.

$$F = \frac{[D^+]K_{a1} + [D^+]^2}{[D^+]K_{a1} + [D^+]^2 + [D^+]K_{a1}K_T(1 + K_t) + K_{a1}K_{a2}K_T(1 + K_t)} \quad (3\text{-}5)$$

$$F \approx \frac{[D^+]^2}{[D^+]^2 + [D^+]K_{1app} + K_{1app}K_{2app}} \quad (3\text{-}6)$$

Reduced eq 3-6 has the same form as that for the titration of a diprotic acid in which the fraction of the total amount of acid in the final diprotonated state is expressed. On computer fitting both values turn out to be about the same, 5.48 and 5.57, for pK_{1app} and pK_{2app}, respectively.

Unfortunately, this second analysis is flawed. (1) At the start of the titration the substrate was said to exist as a mixture of aminal monocations [10]. (2) Our estimate from the computer fit for the pK_a value (5.57) of the conjugate acid of 3-6 is not reasonable if $K_{2app} = K_{a2}/(1+K_t)$ or approximately K_{a2}. The calculated pK_a value for K_{a2} is much too low. The pK_a (H_2O) of the conjugate acid of the model aminal 2-isopropyl-1,3-diethylimidazolidine is 8.42 at 35 °C [45]. (3) The standard deviation of K_{a2} is an unsatisfactory 57% of the equilbrium value. The first scheme based on a single degree of deuteronation is most likely the better model.[5]

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[5] Dideuteronated aminal is not likely to be present in significant amounts [41, 45]. Our superficial analysis based on this dication can be made to fit the experimental data when a step for the direct conversion of this ion to 3-8 is included. Both ring cleavage and proton transfer then are required, perhaps by the participation of a water bridge.

Enamine 3-5 must be only one of several rapidly interconverting structures, its presence being inferred by the observation of hydrogen-deuterium exchange at the beta position of the enamine [10]. The minor contribution of 3-5 also is consistent with the high basicity of enamines [40]; it is expected to be an unimportant contributing structure except in highly alkaline solutions.

Discussion

Skewing of Observed pK_a Values by Concurrent Ring-Chain Equilibria

The presence of concurrent tautomeric and acid/base equilibria during the titration of the oxidation product of spermine and spermidine gives an apparent pK_a which is skewed relative to the true value. Taking the pD value at the midpoint of the titration curve (Figure 3-6) as being equal to a pK_a value provides an apparent pK_a (pK_{app}) of 5.2. Is this value consistent with the structural assignments or does it invalidate them?

On first consideration this would seem to be a pK_a value for the mixture of secondary and tertiary amino groups in the conjugate acid of 3-7, a dication. Comparison of this pK_a value with that of a model compound initially seems to be consistent. Aminals are hydrolytically labile [41] and so we

make comparison not with a desired gem-diamine as in 3-7 but rather with a vic-diamine, as its dicationic acid, e.g., the diprotonated form of 1,2-diaminoethane which has as its first pK_a (H_2O) a value of 7.4 [46]. The diprotonated form of aminal 3-7 would be expected to have a pK_a value much lower than this owing to the larger acidifying effect of the more closely situated ammonio group [41, 45] in apparent agreement with the assignment.

This superficial analysis is not correct, however. The reported titration curve not only applies to a deuteronation step but also to a ring cleavage reaction, a ring-chain tautomeric equilibrium between aminal 3-7a deuteronated at its secondary amino group and its monocyclic aminoalkyl iminium ion 3-9. This equilibrium provides a major perturbation on the true pK_a value. K_{app} then is the product of an equilibrium constant for a tautomeric step (K_T) and an equilibrium constant (K_{a1}) for a weak acid in which the conjugate base of this acid is disfavored by the prior ring-chain equilibrium. The large K_T term skews the apparent acidity constant of the ammonium ion making it large. The apparent acidity constant is not a value for dissociation alone as a simple consideration of the data might first suggest.

Consideration of the pH-dependent hydrolysis profile of 3-3 yields the same result. The inflection point of the titration curve gives an apparent pK_a value of 11.4. This value is skewed relative to the true values due to

simultaneous hydrolytic equilibrium. The observed inflection point (pK_{app}) of 11.4 in the reported titration curve for 3-3 is nicely reproduced by considering the pK_a of the open-chain amine and K_H, i.e., $pK_{app} = pK_{a3} - \log(K_H/(K_H + 1)) = 11.4$ where $pK_{a3} = 10.3$ and $K_H = 0.085$. A similar result was found for anabaseine and was discussed in Chapter 2. Thus, care must be taken when interpreting titration curves for substances which undergo ring-chain interconversion as the apparent pK_a values may not reflect the true pK_a values but instead represent mixtures of equilibrium constants.

Myosmine and Iminium Ion Acidities

Because of the expected interest in the heretofore undescribed variation in the composition of reaction mixtures of 3-1 by those engaged in pharmacological studies, a plot is given in Figure 3-9 showing how the fractional amounts of its four components change with pH. These curves were constructed using the equilibrium constants in Table 3-1 after making a reduction of 0.5 in pK_a values to reflect the change from D_2O to H_2O. Equations such as that given by eq 2-5 describe how the fractional amount of a given component varies with pH.

The titration curve for 3-1 is similar in shape to that of 2-1 and their K_H values also are alike, 1.9 vs 1.2, respectively. However, comparison of the K_{a1} values for the two substances produces a major surprise. The pK_a value of

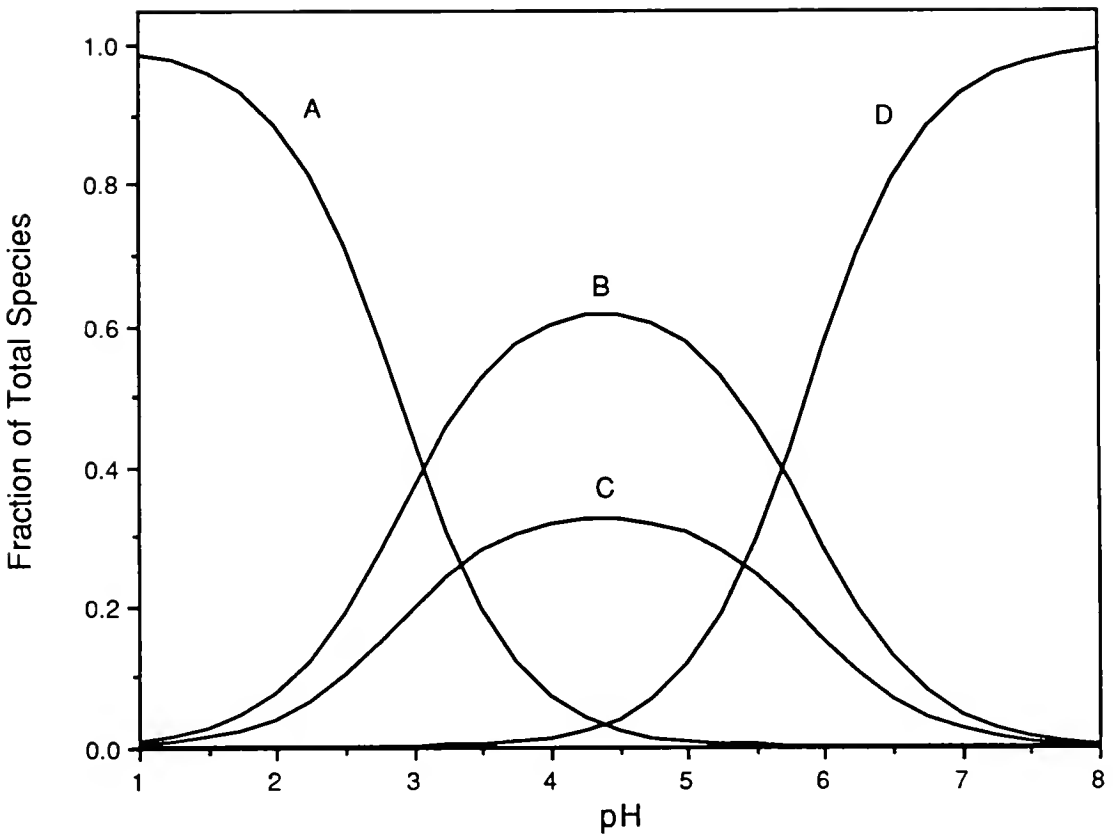

Figure 3-9. Calculated changes in the composition of aqueous solutions of myosmine (3-1) based on the model in Figure 2-1 as a function of pH. The pK_a values in Table 3-1 were decreased by 0.5 to correct for solvent isotope effect. Curves A and B refer to the fraction of amino ketone dication and monocation, respectively. Curves C and D refer to the fraction of protonated cyclic iminium ion and the imine free base, respectively.

5.9 for the smaller ring is very much less than the value of 7.7 for the larger ring. This lower value serves to change markedly the populations of the various components. For example at pD 7 there is 80% of 3-1, 7% of its conjugate acid and 13% of monoprotonated, ring-opened amino ketone while for anabaseine there is 8% of 2-1, 42% of its conjugate acid and 50% of monoprotonated amino ketone. In H_2O these values are

likely to be 93%, 2% and 5%, respectively, for myosmine, Figure 3-9. Much more of the cyclic free base is present compared with 2-1 under the same conditions where 21% imine free base, 36% cyclic iminium ion, and 43% mono-protonated keto ammonium ion are present.

The remarkably large difference in pK_a values for the 5- and 6-membered iminium ions stands in marked contrast to the insignificant difference in pK_a values for the corresponding saturated, secondary amines, pyrrolidine (pK_a 11.13) [47] and piperidine (pK_a 11.07) [48]. This large variation certainly is not due to the different ionic strengths used in the two studies, the former not being reported but is likely to be less than our own.

Two other reports support our claim that the pK_a values of 5- and 6-membered iminium ion acids are very different. (1) A large difference in pK_a values (H_2O) had been reported much earlier for anabaseine (6.7) and myosmine (5.5) from classical titrations [34]. (2) Another old study on similar, simpler structures also has gone largely unrecognized, possibly because the initial structures were assigned their incorrect tautomeric enamine forms. We know today that enamines of secondary amines really exist as imines so that the correct forms of 3-10 and 3-11 are the tautomers [49, 50] 3-12 and 3-13, respectively (eqs 3-7 and 3-8). The pK_a value for the conjugate acid of 3-12 is 9.55 [51] and for 3-13 it is 7.91 [52]. Again there is a large difference and again the acid with the 5-membered ring is stronger.

The early workers recognized but denied the possibility of hydrolysis during their titrations of 3-12 and 3-13. Considering the effect of the 2-methyl group on the value of K_H as discussed below, this assumption may be largely correct. Even if the reported pK_a values are not for a pure substance but are for an equilibrium mixture that is somewhat skewed by the presence of more basic amine, the difference is real and highly significant. The amount by which the reported pK_a (pK_{app}) differs from the true value must be about the same for both the conjugate acids of 3-12 and 3-13, since $pK_{app} = pK_a + \log(1 + K_H)$.

3-10 3-12 (3-7)

3-11 3-13 (3-8)

The greater acidity of the iminium ion with the 5-membered ring over that of the 6-membered ring is likely due to the difference in energy between the free bases. The greater s-orbital character of the lone pair associated with the smaller interior bond angle of the 5-membered ring provides a larger stabilization thereby making the nitrogen

atom less basic. While the direction of the stabilization is expected, the large magnitude is a surprise and raises the question of whether other cyclic imines of varying ring size show similar hybridization effects.

K_H Values for Hydrolysis of Cyclic Iminium Ions

Consideration of all the K_H values, Tables 2-1 and 3-1, for the cyclic imines as a function of substituents suggests a trend. As the group at the imino carbon atom is made less electron-withdrawing in the order 3-pyridyl and phenyl there is less hydrolysis. That is, cyclic imine is favored more as the substituent is made more electron-donating, the same kind of electronic effect as found earlier [9]. Similarly, addition of a methyl substituent to the imine nitrogen atom causes the cyclic form to increase in abundance. These limited data should be considered with caution but they suggest that in the absence of steric effects electron-donating groups preferentially stabilize the protonated iminium ion more than the open-chain ketone. But the combined effect of both changes is modest, being only a factor of 22. This conclusion also suggests that the old pK_a values reported for the conjugate acids of 3-12 [51] and 3-13 [52] may be skewed only a little by the presence of open-chain hydrolysis product because only a little may be present.

Conclusions

Much information can be gained from titration curves for materials which undergo pH-dependent ring-chain equilibria. Construction of these curves is relatively simple and only requires measurements of the relative amounts of the species in equilibrium over the pH range where a change in the composition occurs. With the appropriate model system, the pH-profile can be simulated and relevant equilibrium constants can be extracted.

By varying the pH of physiological experiments with cyclic imines from about 6 to 8 the following predictions may be made with the important assumptions that pH changes in solution are mirrored at the receptor site and that variations in receptor properties due to pH may be corrected by comparison with, say, carbamylcholine. The concentration of anabaseine free base over this interval will increase by a large factor of about 30 while the concentrations of its monocationic cyclic and acyclic ions will decrease by a modest factor of approximately 3. For myosmine over this same pH interval the cyclic free base concentration will increase only by about 2-fold and the concentrations of the cations will fall by about a large factor of 50. Fortunately, the observations for these two substances reinforce each other because there is a major change in composition of different species as the pH is varied, the

neutral form of anabaseine and the monoprotonated entities for myosmine. It therefore should be possible from the increase or decrease in bioactivity to state whether it is the neutral or cationic form that is responsible for promoting activity at the receptor site. However, because both the amounts and pH dependence of the monocations are so similar, it will be necessary to employ non-interconverting model compounds to distinguish between them[6] [53]. In this way it should be possible for the first time to identify both the structure and the state of protonation of these interesting, old neurotoxins when bound to the active site[7] [54].

The identity of the chemically produced oxidation product from spermine and spermidine and of the plant polyamine oxidase product from spermine now has been firmly established. Under physiological conditions the major form is 3-7. The titration data are consistent with the proposed structures.

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[6] Previous analyses of nicotine action upon the neuromuscular acetylcholine receptor as a function of the external pH have provided strong evidence that the monocationic species is much more active than the neutral form [53].

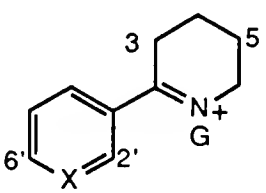
[7] A comparison of the activity of 2-1 and 3-1 has been made with insects, 3-1 being less reactive [54].

CHAPTER 4
COMPENSATORY CHANGES IN THE INFLUENCE OF COSOLVENTS ON THE POSITION OF THE RING CHAIN EQUILIBRIUM FOR ANABASEINE AND N-METHYLANABASEINE

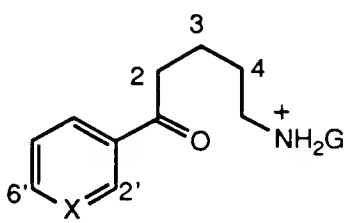
Introduction

Because the nicotinic receptor for cholinergic agents may be in a hydrophobic environment [55 - 57], studies were designed to determine what influence a hydrophobic environment might have on the equilibrium ratio for anabaseine (4-1), its derivatives and analogues. The positions of the ring-chain equilibria of 4-1 with 4-2 and of its N-methyl derivative 4-3 (1-methyl-3,4,5,6-tetrahydro-2,3'-bipyridinium ion) interconverting with 4-4 were measured in aqueous and nonaqueous solvents and their mixtures by ^{1}H NMR [12]. First, a general survey of the solvent effects of water, DMSO, and methanol on the equilibrium compositions of solutions of 4-1 and 4-3 is presented. This is followed by a quantitative study of the changes in the equilibrium compositions in water-methanol and water-DMSO. The compositions of solutions of 4-1 and 4-3 in water-methanol mixtures can be described by a linear free energy relationship.

Analogues 4-3 and 4-5 (1-methyl-6-phenyl-2,3,4,5-tetrahydropyridinium ion [58]) were prepared so that the effects of methyl substitution on the hydrolysis of the 6-membered imine rings could be compared with those of the 5-membered cyclic imines discussed in Chapter 3. Analogue 4-5 has a phenyl substituent in place of the 3-pyridyl ring and hydrolyzes to 4-6. N-Methylated 4-5 was included to further substantiate the influence of a steric effect in the N-methyl compounds on the position of the ring-chain equilibrium.



4-1, X=N, G=H
4-3, X=N, G=CH_3
4-5, X=CH, G=CH_3



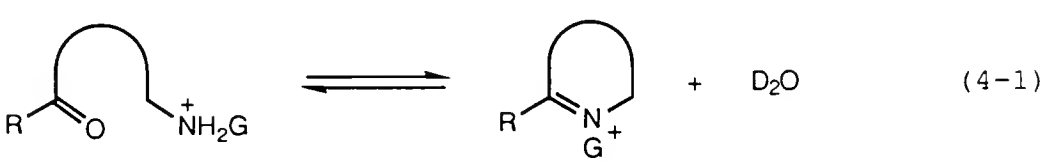
4-2, X=N, G=H
4-4, X=N, G=CH_3
4-6, X=CH, G=CH_3

Claisen-type syntheses of the known compounds 4-1 [59], 4-3 [58], and 4-5 [58] gave the desired substrates and will be discussed in Chapter 6. The nonaqueous model solvents include two that are moderately polar, amphiprotic CD_3OD and the more basic dimethyl sulfoxide-d_6, a strong hydrogen bond acceptor [60 - 62].

Results

Ring-Chain Equilibria and Nomenclature

A question arises as to how to name the solid compounds used as starting materials. Elemental analyses indicate they contain one equivalent of water in the solid state; the associated structures are compatible either with a ketone or with an imine containing a stoichiometric amount of free water in the lattice (water of hydration). Which structure is correct and therefore which name should be designated? This is a classical question that could be answered by "magic angle" solid state NMR but it was not addressed because we are interested in the solution chemistry. Further confusion arises about how to indicate the degree of protonation of N-methylated substrates in the solid state because, for example, the conjugate acid of ketone 4-4 is diprotic (a dihydrohalide) while that for iminium ion 4-3, its equilibrium component, is monoprotic (a monohydrohalide). Equation 4-1 schematically illustrates the stoichiometry for the hydrolysis reaction, showing the equilibrium between the cyclic and acyclic forms where G is H or CH_3.



(4-1)

We have elected to name our starting materials using the common name for the cyclic imine as have others [58] and therefore designate its associated degree of protonation. The systematic name for the open-chain amino-ketone is not employed in spite of the possibility that in the solid state the predominant form is acyclic. Thus, we prefer the common name N-methylanabaseine cation (4-3) over the cumbersome name for its hydrolysis product, 5-methylammonio-1-(3-pyridyl)-1-pentanone (4-4). Hence, salts of 4-3 will be referred to schematically as 4-3.Cl for that cyclic chloride having an unprotonated pyridine ring while 4-3.Cl.HCl denotes its conjugate acid, the pyridinium salt. Similarly, the dihydrobromide of 4-1 is designated 4-1.2HBr and the monohydrobromide 4-1.HBr.

Survey of Solvent Effects

Preliminary studies first show the scope of solvent changes on the position of the equilibrium [63]. Then a quantitative investigation with mixed water-methanol solvents along with a more limited study of water-dimethyl sulfoxide mixtures is described.

(1) Water

N-methylated 4-3.Cl.HCl gave a solution with a pD of 3.0 where the material exists largely as the dication. The only form present was the conjugate acid of open-chain amino ketone 4-4. A solution of 4-3.Cl gave a pD of 6.0 and two

separate solutions of 4-3.Cl.HCl made basic with carbonate gave pD values of 9.2 and 9.6. In these three cases approximately 6-7% of the cyclic iminium ion 4-3 appeared, Table 4-1. The ratio of open-chain keto-ammonium ion to cyclic iminium cation under neutral conditions thus is about 14. The assignment of structures was made by comparison with the proton NMR shifts we previously observed for the non-methylated derivatives 4-1 and 4-2 under similar conditions [Chapter 2].

Anabaseine dihydrobromide exists largely as the amino ketone while 4-1.HBr has nearly equal amounts of the two forms, the ratio of the open-chain to cyclic structures being about 16 and 1.3, respectively. Phenyl derivative 4-5 exists largely as open-chain protonated amino ketone; there is 3.5 times more ketone than iminium ion, Table 4-1.

(2) DMSO

NMR spectra of 4-1 and 4-3 either as their dihydrohalide or monohydrohalide salts were recorded using DMSO-d_6 over a concentration range of 0.04 to 0.16 M. The added solids contain one equivalent of water as indicated by elemental analyses and so an equilibrium exchange of water is possible in the absence of any added water. Chemical shifts for the amino ketones, 4-2 and 4-4, in DMSO-d_6 and CD_3OD appear in Table 4-2 and chemical shifts for cyclic imines, 4-1 and 4-3, and enamine, 4-7, appear in Table 4-3.

Table 4-1. Composition of Ring-Chain Equilibrium Mixtures in Various Solvents Determined by ^1H NMR at 22° C.

Compound	M	Solvent	Percent Ketone	Imine	Other
4-1.2HBr		D_2O	94	6	0
	0.085	DMSO-d_6	100	0	0
	0.071	CD_3OD	72	19	9[a]
	0.071	CD_3OD	32	42	26[a,b]
4-1.HBr	0.080	D_2O	57	43	0
	0.079	CD_3OD	trace	100	0
	0.075	CD_3OD/D_2O	27	73	0[c]
4-1.HCl	0.080	DMSO-d_6	10	90	0
	0.074	DMSO/D_2O	76	24	0[d]
	0.071	DMSO/D_2O	78	22	0[e]
4-3.Cl.HCl	0.17	D_2O	100	0	0
	0.053	D_2O/Na_2CO_3	94	6	0[f]
	0.039	D_2O/Na_2CO_3	93	7	0[g]
	0.036	DMSO-d_6	97	3	0
	0.068	CD_3OD	65	26	9[a]
	0.083	CD_3OD	86	5	9[a]
4-3.Cl	0.13	D_2O	93.5	6.5	0
	0.043	DMSO-d_6	60	21	19[h]
	0.082	DMSO-d_6	72	23	5[h]
	0.16	DMSO-d_6	78	16	6[h]
	0.078	DMSO/D_2O	100	trace	trace[c,h]
	0.087	CD_3OD	34	66	0
	0.080	CD_3OD/D_2O	77	23	0[c]
4-5.Br		D_2O	78	22	0

[a]Unknown structure.
[b]Same sample as above but after 10 days.
[c]2.6 M D_2O.
[d]4.1 M D_2O.
[e]5.9 M D_2O.
[f]pD=9.62, 70 mM Na_2CO_3, 0.5 M NaCl.
[g]pD=9.20, 42 mM Na_2CO_3, 0.5 M NaCl.
[h]Enamine.

Table 4-2. Chemical Shifts (δ/ppm) for Amino Ketones 4-2 and 4-4 in Nonaqueous Solvents.

Solvent	Compound	H2'	H6'	H4'	H5'	H2	H3, H4	H5	NCH_3
DMSO-d_6	4-2.2HBr	9.38	9.05	8.79	8.02	3.25	1.70	2.88	--
	4-4.Cl.HCl	9.31	8.97	8.65	7.90	3.23	1.72	2.94	2.53
	4-2.HBr	9.17	8.82	8.35[a]	7.60	3.17	1.68	2.86	--
	4-4.Cl	9.17	8.82	8.34	7.60	3.17	1.70	2.91	2.51
CD_3OD	4-2.2HBr	9.43	9.06	9.16	8.26	b	1.84	3.01	--
	4-4.Cl.HCl	9.42	9.06	9.15	8.26	b	1.88	3.07	2.71
	4-2.HBr	9.11	8.73	8.40	7.58	b	1.79	2.98	--
	4-4.Cl	9.12	8.73	8.41	7.58	3.17	1.80	3.05	2.71

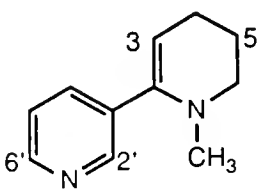
[a] Overlaps imine H4'.
[b] Overlapped or exchanged.

Table 4-3. Chemical Shifts (δ/ppm) for Cyclic Imines 4-1 and 4-3 in Nonaqueous Solvents.

Solvent	Compound	H2'	H6'	H4'	H5'	H3	H4, H5	H6	NCH_3
DMSO-d_6	4-3.Cl.HCl	a	8.90	8.25	7.78	a	2.01, 1.87	3.94	3.46
	4-1.HBr	9.10	8.90	8.35[b]	7.70	3.28	1.91[c]	3.81	--
	4-3.Cl	8.90	8.84	8.16	7.70	a	2.01, 1.89	3.93	3.44
CD_3OD	4-1.2HBr	9.32	a	8.79	8.08	d	2.07[c]	3.95	--
	4-3.Cl.HCl	9.20	a	8.71	8.14	d	2.16, 2.02	4.05	3.56
	4-1.HBr	9.02	8.85	8.32	7.68	d	2.02[c]	3.88	--
	4-3.Cl	8.8[a]	8.8[a]	8.12	7.68	d	2.13, 2.01	4.00	3.52
$CDCl_3$	4-7	8.63	8.48	7.69	7.26	5.03	2.16, 1.78	3.09	2.42

[a] Overlapped.
[b] Overlaps ketone H4'.
[c] H4 and H5 overlap.
[d] Overlapped or exchanged with deuterium.

Results summarized in Table 4-1 show (i) dications exist almost entirely as their open-chain keto ammonium ions, (ii) monocations give more of the cyclic iminium ions, iminium ion being the major product from 4-1 and a minor product from 4-3. In addition, the enamine 4-7 (1-methyl-1,4,5,6-tetrahydro-2,3'-bipyridine) is clearly detectable from the monohydrohalide of 4-3. Formation of the enamine under such mild conditions is remarkable. By contrast, in aqueous solution the addition of base and a high pH is required for enamine formation [9].



4-7

An authentic sample of the enamine derivative 4-7 was generated by extracting with chloroform an aqueous solution of 4-3 made alkaline with sodium carbonate. Its NMR spectrum contains among other signals a triplet due to the alkene proton at 5.03 ppm as proof of its structure.

Varying the concentrations of 4-3.Cl causes the product ratio to change. More dilute solutions give rise to lower concentrations of ketone and consequently more iminium ion and more of its conjugate base, the enamine 4-7. The ratio of ketone to the total amount of iminium ion and enamine decreases from 3.5 to 2.6 to 1.5 on diluting substrate.

Both the presence of enamine and its increasing contribution with dilution is readily understandable. In an equilibrium such as that given by eq 1 where unequal numbers of particles are involved, dilution favors that side having the larger number of particles, in this case the iminium ion and water. On dilution the solution becomes less acidic as the keto ammonium ion acid is converted to the weaker acid water. This decrease in medium acidity causes more of the N-methyl iminium ion to dissociate to its conjugate base the enamine 4-7. The halide counter ion although substantially more basic in DMSO than in water is not likely to serve as the active base for deprotonation. Instead it is the more abundant solvent. Adding 5 volume percent D_2O to 4-3.Cl shifts the equilibrium almost completely to ketone as expected from mass action considerations.

(3) Methanol

Samples about 0.08 M in CD_3OD show the following characteristics, Table 4-1: (i) dications exist largely as open-chain ketones but substantial amounts of cyclic iminium ions are present as well, (ii) monocations exist mostly as the iminium ion, more being favored from 4-1 than from 4-3, (iii) adding 5 volume percent D_2O to the monocations causes more ketone to form and (iv) both dications give <10% of an unknown material which may be a cyclic hemiaminal [64] or an acyclic ketal. The structure was not identifiable because

some of the high field peaks were overlapped by the major components.

Quantitative Studies

(1) Water-methanol

Serial dilution experiments were performed with 4-1.HCl and with 4-3.Cl in the mixed solvent. To a methanolic solution of each substrate was added a measured amount of D_2O and the NMR spectrum of the mixture was recorded to provide the equilibrium composition. As expected from Table 4-1 the addition of water causes the amount of acyclic ketone to increase. This increase occurred rapidly at first with small additions of water and then more slowly. The smooth increase in the ratio of the concentrations of acyclic keto ammonium ion 4-2 to cyclic iminium 4-1 is shown in Figure 4-1 and similarly for the ratio of 4-4 to 4-3 in Figure 4-2 as the mole fraction of water increases to about 0.45.

A linear free energy relationship (LFER) quantitatively describes the change in the ratio of the two forms of the two substrates as the composition of the solvent is varied and provides the two desired equilibrium constants for the pure solvents water and methanol, eq 4-2.

$$\ln K_{mix} = F_D \ln K_D + (1 - F_D) \ln K_M \qquad (4\text{-}2)$$

$$\ln K_{mix} = F_D \ln (K_D/K_M) + \ln K_M \qquad (4\text{-}3)$$

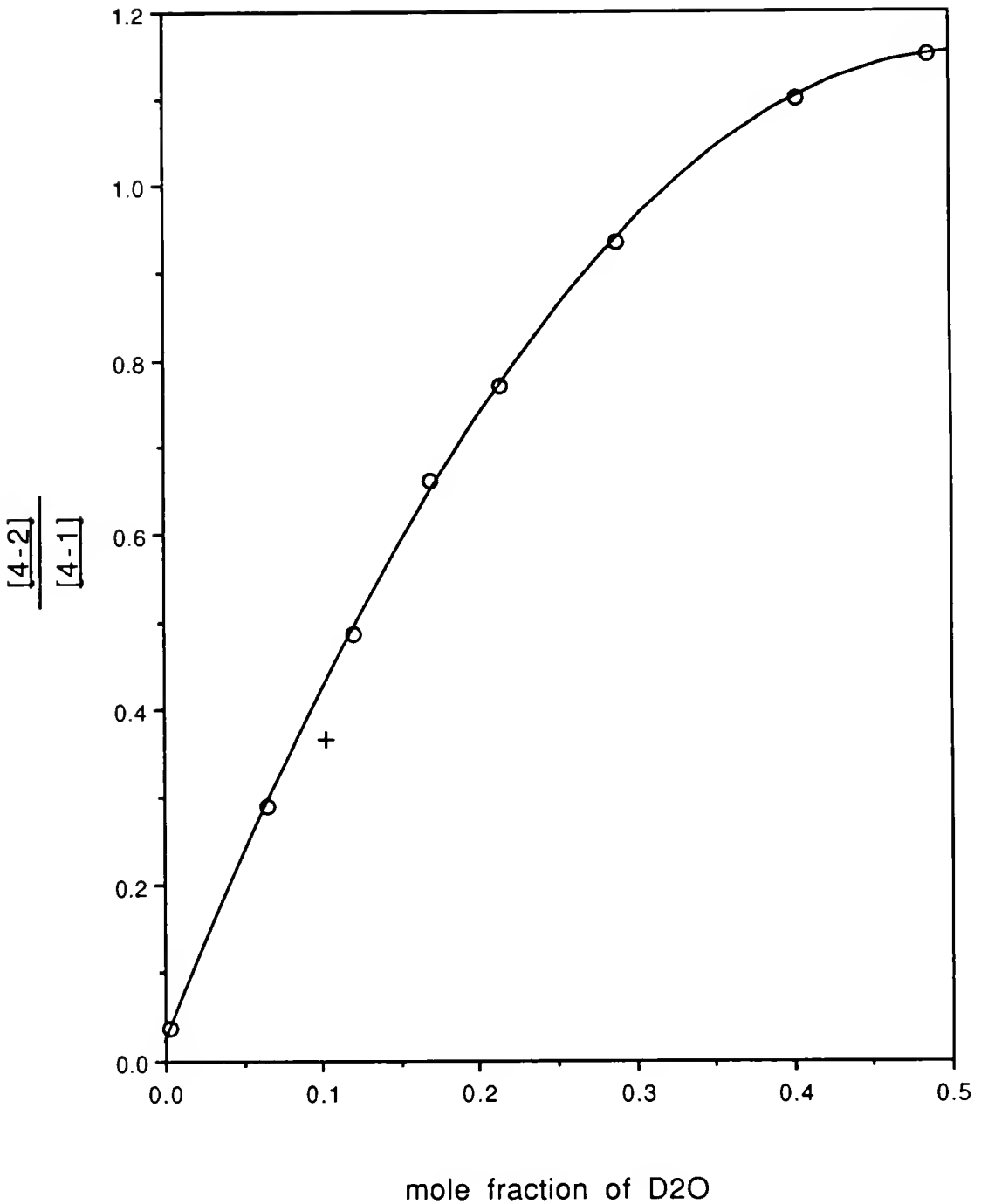


Figure 4-1. Observed ratio of concentrations of keto ammonium ion, 4-2, to iminium ion, 4-1, as a function of mole fraction of D_2O in CD_3OD at 22 °C and uncorrected for "impurity" of water in methanol. The cross denotes the result of a separate determination.

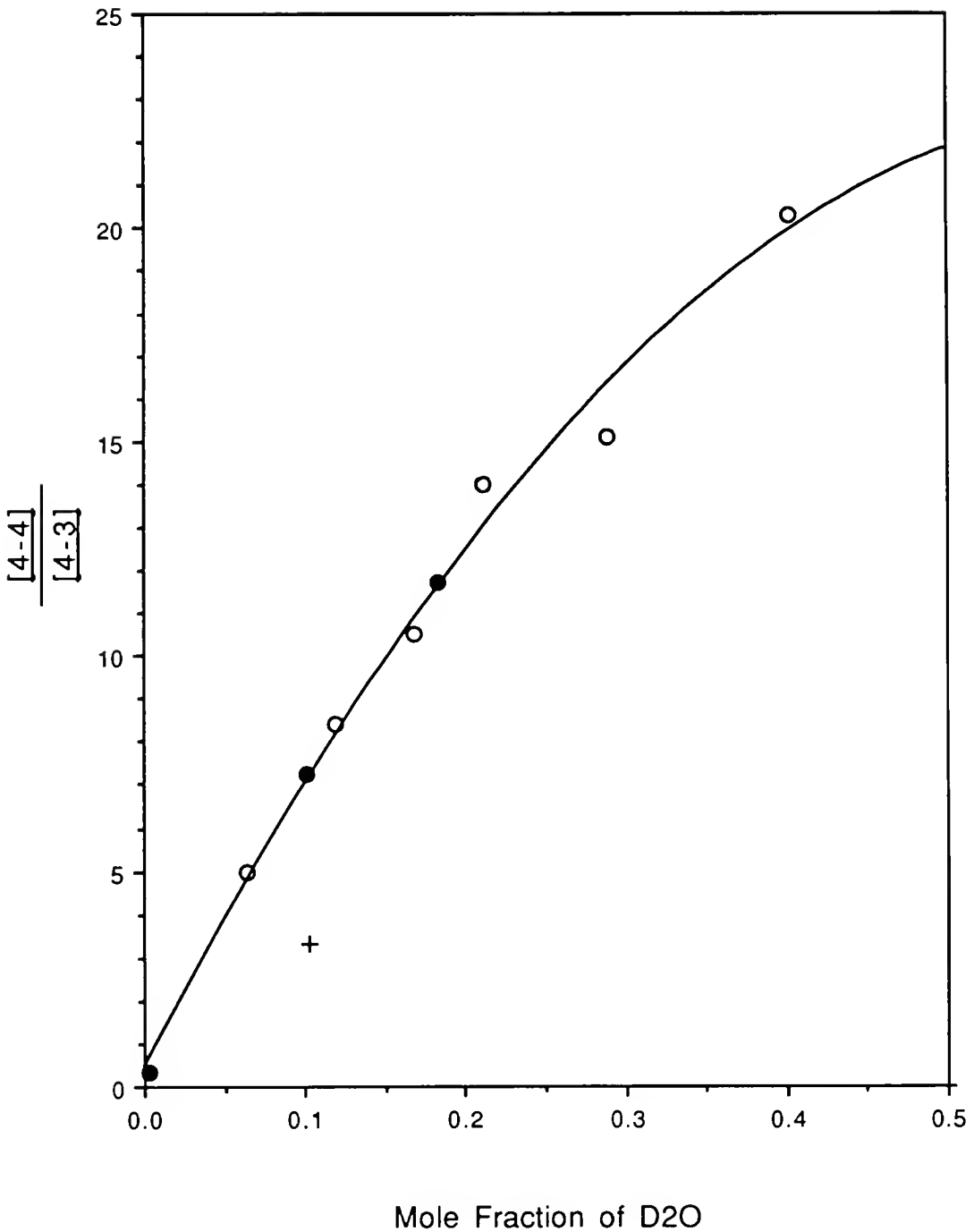


Figure 4-2. Observed ratio of concentrations of keto ammonium ion, 4-4, to iminium ion, 4-3, as a function of mole fraction of D_2O in CD_3OD at 22 °C and uncorrected for "impurity" of water in methanol. Open and filled circles are due to two separate serial dilution experiments. The cross represents a separate determination.

In the LFER given by eq 4-2, K_{mix} represents the observed equilibrium constant for the mixed solvent, K_D denotes the equilibrium constant when pure D_2O is the medium, K_M is that for pure CD_3OD, F_D is the mole fraction of deuteriated water and $(1 - F_D)$ is the mole fraction of CD_3OD in the mixture. The equilibrium constant is defined as [ammonio ketone]/([iminium ion] $[D_2O]$) or $K = [AK]/[Im][D_2O]_{tot}$ where $[D_2O]_{tot}$ denotes the total amount of water from all sources as explained below. The LFER actually was applied using rearranged eq 4-3 and the outcome is presented graphically in Figures 4-3 and 4-4 for 4-1 and 4-3, respectively. An equation similar to eq 4-2 has been reported, for example, for rates of reactions in mixed solvents but in these cases, unlike our own, one of the solvents was not a reactant [65].

Calculation of the mole fraction F_D and the concentration of water in a mixture requires some care because two minor corrections are needed. They are based on the following considerations: (i) a correction needs to be applied to reflect the release of water into the medium when the ketone is converted to iminium ion, eq 4-1 and (ii) the commercial sample of CD_3OD usually was not dried and the initial amount of water "impurity" in the methanol was not determined independently. Both corrections are made easily. In the first the observed equilibrium quantities of ketone and imine provide a measure of the amount of water liberated when ketone cyclizes. In the second the unknown quantity of "impurity" water is treated as an adjustable parameter

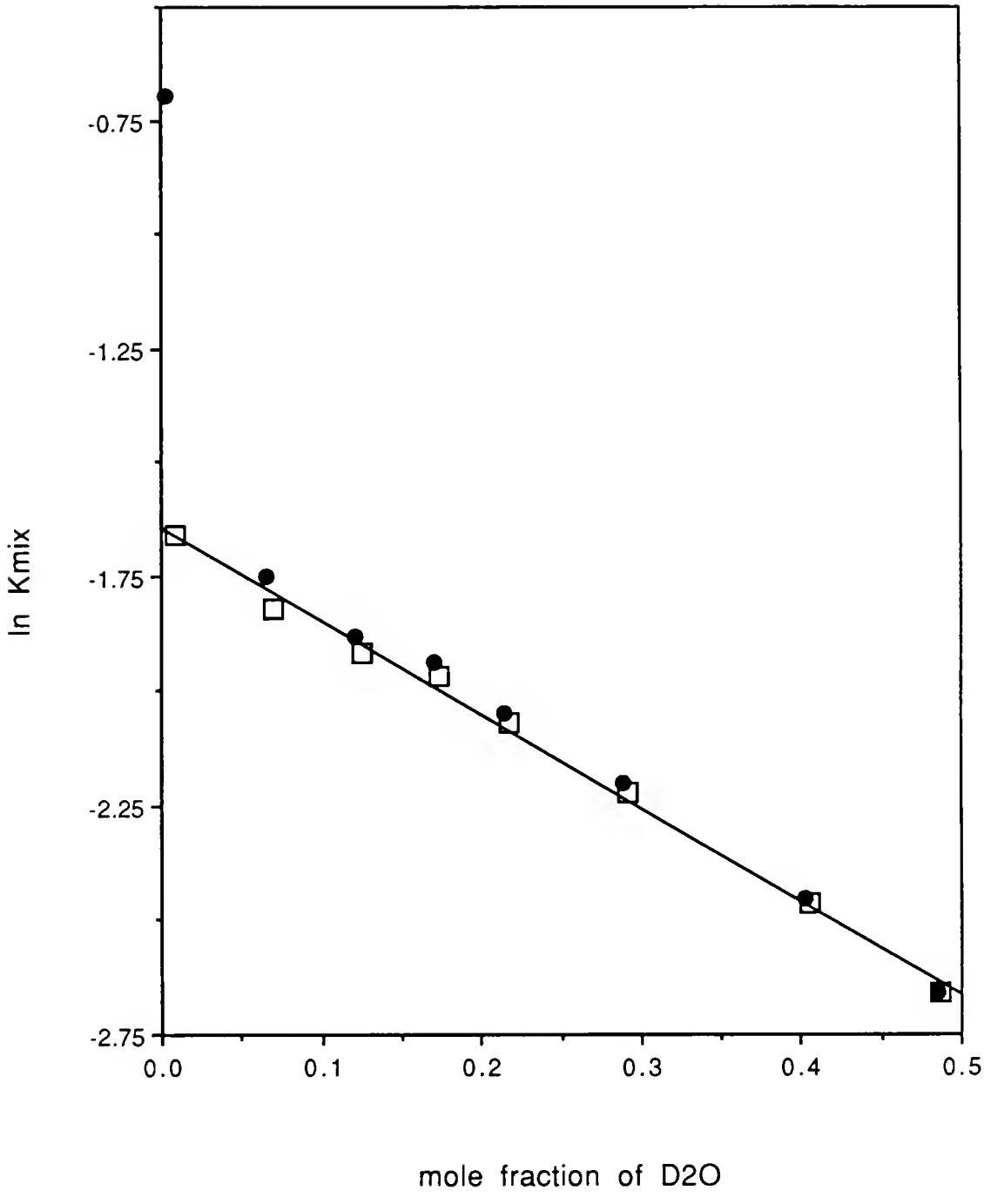


Figure 4-3. Linear relationship between ln K_{mix} verses mole fraction of D_2O in CD_3OD at 22 °C where K_{mix} = [4-2]/ ([4-1][D_2O]). The filled circles represent the actual data points uncorrected for the amount of "impurity" water present in the solvent while the open squares include this adventitious water calculated according to eqs 8-3 and 8-4. The slope is -2.02 and the intercept, K_M, is -1.65.

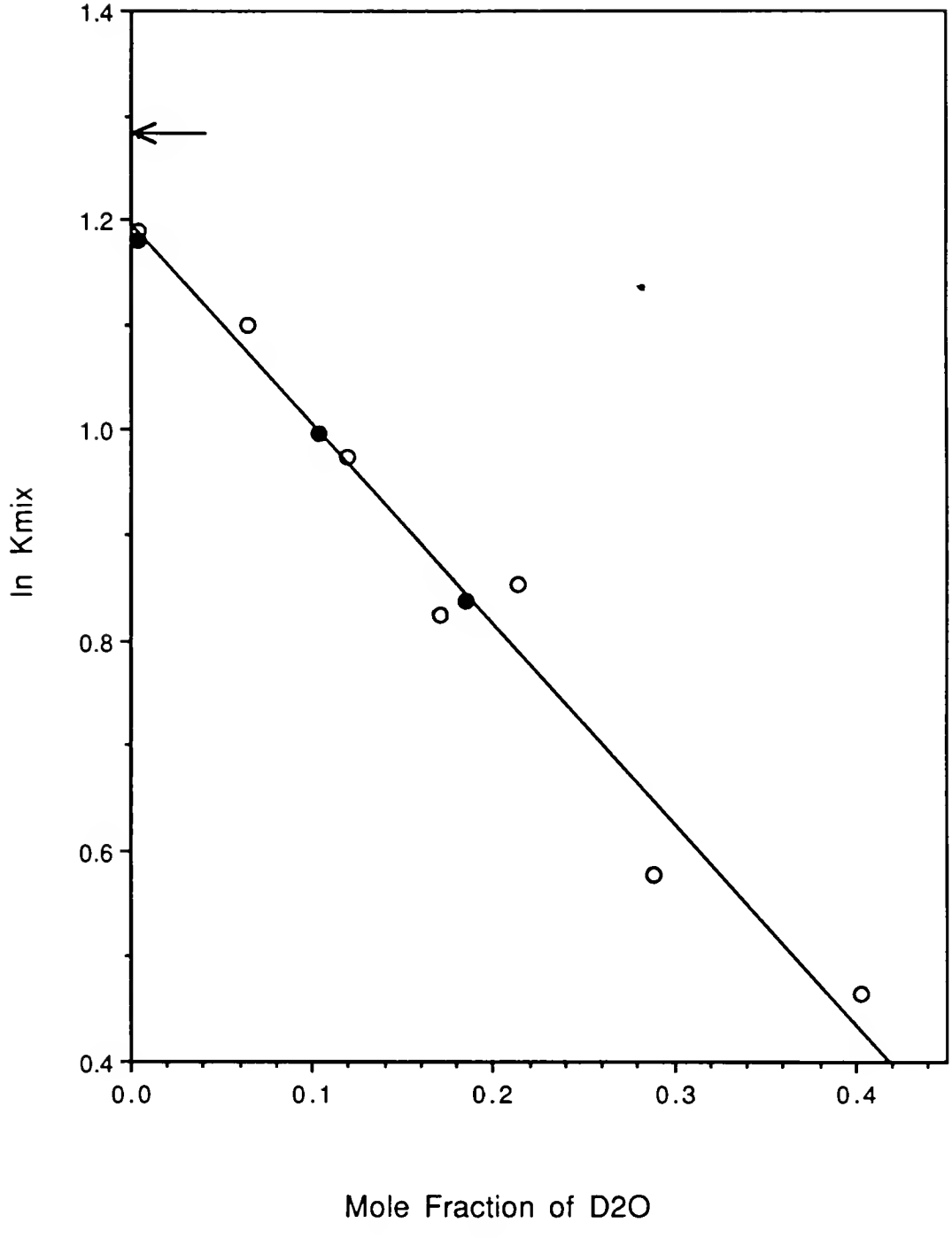


Figure 4-4. Linear relationship between ln K_{mix} verses mole fraction of D_2O in CD_3OD at 22 °C where K_{mix} = [4-4]/([4-3][D_2O]). The slope is -1.90 and the intercept, ln K_M, is 1.20. Open and filled circles represent two separate serial dilution experiments while the arrow denotes the value of the equilibrium constant obtained using solvent dried with molecular sieves.

thereby avoiding the need for tedious drying and determination of the actual water concentration. Determination of the amount of water impurity in the solvent is achieved by a computer fitting of all the experimental data based on eq 4-3 as described in the Experimental Section [Chapter 8]. These are minor corrections and only materially affect K_{mix} when the measured amount of added water is small but they provide gratifyingly improved fits of the data.

The LFER given by eq 4-3 first was rearranged and then the data were fit using a nonlinear regression microcomputer program that yielded the desired equilibrium constants as well as the the amount of impurity water. The exact form of the equation is given in the Experimental Section [Chapter 8]. Figure 4-3 shows a typical set of data points with and without the correction for water impurity; there is only one value that changes substantially.

Having a measure of the total water concentration allows the construction of a plot of eq 4-3 to show clearly the influence of water on the apparent equilibrium constant. Accordingly, the intercept gives K_M while the product of the antilog of the slope and the antilog of the intercept provides K_D. The K_D and K_M values for 4-1 are 0.025 and 0.19 M^{-1} respectively (correlation coefficient, r, for eq 4-3 is 0.997) and for 4-3 they are 0.49 and 3.3 M^{-1}, respectively (r, 0.984). For 4-1 the value of K_D determined in earlier [Chapter 2] using purely aqueous solutions is 0.022 M^{-1} (K_H/55.3 M), which is in very good agreement with the present

value. There is more scatter in the data for 4-3, Figure 4-4. The ratio of ketone to imine is so large that the uncertainty in the measurement of the minor component is sizable. Thus, for example, the K_D value for 4-3 from the LFER is 0.49 M^{-1} while the experimental value measured directly is 0.26 M^{-1} (93.5/(6.5 x 55.3)). However, in the former case this corresponds to a composition of 3.6% imine, the latter to 6.5%, two values the same within the experimental uncertainty of our measurements. The computer fit indicates that the amount of water initially present in the methanol is 0.13 and 0.036 M for 4-1 and 4-3, respectively. Contributing to the quality of the fit is the expectation that the pK_a values for the nitrogen acids will be similar in the two solvents [66, 67] and that no significant change in the fraction of protonated material occurs in the mixtures. Only small variations in the chemical shifts of the substrates were observed in support of this suggestion.

The two linear relationships given in Figures 4-3 and 4-4 may be interrelated through their common axis, mole fraction water. Thus, $\ln(K_{mix})^{4-1} = 1.06\ln(K_{mix})^{4-3} - 2.93$ showing that both 4-1 and 4-3 respond in very nearly the same way to additions of water to methanol.

As a check on our computational approach to determine and to correct for the amount of adventitious water present in "dry" methanol two experiments were carried out following drying of the solvent with 3A sieves. Only when elaborate

attempts were employed in the second of these to exclude moisture, including drying of the glassware, was it possible to obtain an equilibrium constant K_M having essentially the same value (3.6 M^{-1}) as that obtained from the LFER (3.3 M^{-1}). Moreover, in this case enamine 4-7 also was detected in about 6% yield. Clearly the additional effort required to dry the methanol is unnecessary; trace amounts of water may easily be established using our serial dilution technique and computer fitting of the data as given by eqs 8-3 and 8-4 in the Experimental Section.

In addition some other new samples were prepared independently in the usual way without prior drying and the resultant data applied to the curves in the figures in order to check that the curves indeed are reproducible. Figures 4-1 and 4-2 show the new points fit within the uncertainty of the measurements and therefore our results are verifiable. However, a generalization should be kept in mind: as the ratio of components changes from one in value, the uncertainty in the value of the equilibrium constant increases [30] and this accounts for the greater scatter in Figure 4-4.

(2) Water-DMSO

Serial dilution experiments were performed with 4-1.HCl by adding D_2O to samples in DMSO-d_6. Analysis of the three data points, Table 4-1, produced an LFER based on the mole fraction of added water. The value of K_{DMSO} representing the

equilibrium constant in pure DMSO is 1.5 M^{-1}. However, the derived value of K_D for pure water solvent is 0.078 M^{-1}, substantially higher than that observed directly for mesurements made with pure water and also that obtained from methanol-water mixtures. Moreover, in contrast to the spectra obtained using methanol-water samples which did not show significant changes in chemical shift on addition of water, the NMR spectra of the DMSO-water samples contained large changes in shifts for the imine but not for the ketone. Pyridine signals usually moved upfield suggesting that the mixture became less acidic. However, the methylene protons adjacent to the imino nitrogen moved in the opposite direction consistent with increased protonation. As these shifts give inconsistent data about protonation, we made no attempt to interpret them and do not regard the LFER as particularly meaningful.

An estimate was made of the value of K_{DMSO} for 4-3.Cl based on the assumption that all the water from the three entries in Table 4-1 come from substrate when it forms imine and enamine. The derived equilibrium constant has the value 150±13 M^{-1} and represents an upper limit because any water present as an impurity was not considered in the derivation.

Discussion

Solvent Effects

Three different solvents were chosen to study the influence of environment on the position of the ring-chain equilibrium involving the transfer of water to cyclic iminium ion to form acyclic keto ammonium ion, eq 4-1. These include water as the reference and hydroxylic but less polar methanol and polar, protophilic dimethyl sulfoxide. For the positively charged nitrogen acids examined, only small changes in their pK_a values are expected when dissolved in the chosen solvents. DMSO, being more basic than water, will bring about more dissociation than water [60, 62, 68]. The pK_a values for the compounds in methanol and water are likely to be similar [66, 67].

No attempt was made to dry the commercial, perdeuteriated solvents. Moreover, up to one equivalent of free water may be present in the non-aqueous solvents as provided by the heterocyclic salts themselves based on their composition. This stoichiometric water is bound in the amino ketone and is liberated as free water on conversion to imine, eq 4-1. As our quantitative treatment of the data provided by methanol-water mixtures shows, eq 4-3, drying is unnecessary for a systematic study because the amount of

adventitious water can be determined on computer fitting of the data.

Dramatic shifts in the position of the ring-chain equilibrium do occur both as a function of the composition of the starting material as well as of the solvent. While the dihydrobromide of 4-1 exists almost completely as the acyclic ketone in water and in DMSO, it is almost entirely in the cyclic iminium structure when present in methanol as the monohydrobromide. A similar but less extensive shift is found with 4-3.Cl.HCl which is virtually all ketone in both water and DMSO while 4-3.Cl is about 65% iminium ion in methanol, Table 4-1.

Major conclusions derived from the contents of Table 4-1 and the figures are as follows. (1) Ketone is favored in most of the trials listed in Table 4-1. That is, the equilibrium given by eq 4-1 need not be shifted largely to the left favoring iminium ion according to the law of mass action in spite of the concentration of water being low in "anhydrous" methanol and DMSO. (2) Generally the least amount of cyclic iminium ion is present in pure water, (3) more iminium ion is present in methanol than in dimethylsulfoxide and (4) more iminium ion is present in the less acidic solutions, especially when the starting compound is the monohydrohalide rather than the dihydrohalide salt. The latter conclusion is in keeping with our prior observations for purely aqueous solutions [Chapter 2]. Interestingly, although iminium ion and especially neutral

imine are favored by basic conditions, the most basic solvent, DMSO, does not provide the most iminium ion from any salt, showing that the basicity of the medium is not the only relevant factor. Solvation, perhaps by means of H-bonding, especially to stabilize the alkyl ammonium cation, which has a larger number of acidic protons than the iminium ion, should have an important influence on the position of the equilibrium and DMSO is an especially effective H-bond acceptor [68]. (5) The values of K_D for 4-1.HBr and for 4-3.Cl are 0.025 and 0.49 M^{-1}, respectively, and for K_M they are 0.19 and 3.3 M^{-1}, respectively, as derived from Figures 4-3 and 4-4.

The changes in the values of the equilibrium constants can be understood in terms of the activity coefficients for substrate transfer from one solvent to another. According to this, the concentration terms cancel in our ratio of equilibrium constants leaving a ratio of solvent activity coefficients [69, 70] eq 4-4, where ${}^{D}\gamma^{M}$ represents the solvent activity coefficient for transfer from D_2O (D) to CD_3OD (M)

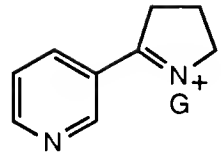
$$K_D/K_M = {}^{D}\gamma^{M}_{AK} / ({}^{D}\gamma^{M}_{Im}\ {}^{D}\gamma^{M}_{D}) \qquad (4\text{-}4)$$

of the component designated by the subscript. Activity coefficients have been reported for H_2O in CH_3OH based on mole fraction with unit mole fraction as the standard state [71, 72]. These values indicate little deviation from ideality. Therefore, our water term appears to account for

very little of the change in the observed equilibrium constant ratio, most of the change being associated with the two organic components. A similar activity coefficient ratio may be written for equilibria in DMSO where again data are available for water-DMSO mixtures. In this instance the value of the solvent activity coefficient for a mole fraction water of 0.1 is 0.28 [73]. Again the major influence of the change in the solvent composition appears to be on the energy of the organic components. Perhaps the energy of the keto ammonium ion is changed more than iminium ion because H-bonding of its ammonio protons to the DMSO solvent are so strong.

Steric Effects

The equilibrium composition of 4-3.Cl in water is not that expected from a consideration of a model compound. Myosmine (4-8, G=H), an analogue of 4-1 and a minor tobacco alkaloid binding to nicotinic cholinergic receptor sites, has a 5-membered 1-pyrroline ring rather than the 6-membered 1-piperideine ring in 4-1 and 4-3. Replacing the hydron bonded to the imino nitrogen atom of 4-8 by a methyl group to give the 5-membered analogue 4-8 ($G=CH_3$) of 4-3 causes a little less ketone ion to form on hydrolysis in D_2O. The ratio of the amounts of open-chain keto ammonium ion to cyclic iminium ion only decreases from 1.9 to 0.88 with this substitution



4-8

on nitrogen because the electron donating N-methyl group preferentially but slightly stabilizes the iminium ion at the expense of the ketone [8, 9, Chapter 3]. Replacing the iminium hydron of 4-1 by a methyl group to give 4-3, in marked contrast, has the opposite effect from that just considered with 4-8. The methyl group causes the iminium ion to become strongly disfavored in the equilibrium, Table 4-1. As given by the ratio of K_D and K_M values, the equilibrium constant for 4-3 is 20 and 17 times larger than that for 4-1 in D_2O and CD_3OD, respectively. Overriding the stabilizing electronic effect of the methyl group is a destabilizing steric effect for the larger 6-membered ring. As the iminium ring is made larger, the methyl group and the adjacent pyridine ring are forced closer together, raising the energy of the cyclic structure and thereby shifting the equilibrium to favor the acyclic material where these two substituents now are separated and no longer can interact. The nominal angle defined by lines drawn from the substitutents to the center of the ring marking the separation between the two annularly bonded groups on a 5-membered ring is 72°; for a 6-membered ring it is only 60°. The smaller angle supports the suggested steric compression between the two adjacent groups on the larger ring.

Making the approximation that the energies of the open-chain keto primary and secondary alkylammonium ions 4-2 and 4-4, respectively, are similar allows the difference in energy between the two cyclic iminium ions to be estimated. Thus, from the ratio of the two equilibrium constants and their average for the two solvents there is about 1.7 kcal/mol (RT ln 18) of steric compression energy in 4-3 that is not present in 4-1, providing a considerable driving force for ring opening.

The same kind of steric effect is also found with phenyl derivative 4-5 [74]. Again, a consideration of model compounds is instructive, this time making changes at the carbon atom of the imine rather than at nitrogen. For cyclic 5-membered iminium 4-8 ($G=CH_3$) replacing the more electron-withdrawing 3-pyridyl ring by a phenyl substituent causes the ratio of acyclic to cyclic ions to decrease about 10-fold due to preferential electronic stabilization of the iminium group by conjugation with the more electron-donating phenyl ring [Chapter 3]. However, the equilibrium composition of pyridyl 4-3.Cl and phenyl 4-5 in water is similar, Table 4-1, contrary to the expectation raised by the model compounds. Steric compression between the N-methyl group and the benzene ring causes the iminium ring to be destabilized, negating the stabilizing electronic effect of the phenyl ring on the iminium ion.

Significance for Binding Studies

The results for 4-1.HBr, 4-1.HCl and 4-3.Cl provide the most useful information about the influence of solvent composition at a hydrophobic binding site on the position of the ring-chain equilibria for these substrates in biological experiments. The acidities of the aqueous solutions in our studies involving these substrates should reasonably approximate those of binding experiments while those for 4-1.2HBr and 4-3.Cl.HCl are greater and therefore less relevant. The composition of 4-1.HBr in D_2O, Table 4-1, is essentially the same (1.3) as that observed with aqueous phosphate buffers where the ratio (1.2) of the concentrations of the open-chain to cyclic forms is almost constant over the pD interval 4.5 to 7.5 [Chapter 2].

Consideration of the results given in the figures is quite informative about the effect of the change in water content of methanol-water mixtures on the ring-chain ratio. But first it is necessary to understand what is meant by the definition of K_M in eq 4-2 and 4-3 for an equilibrium involving the transfer of water between two substrates in a non-aqueous solvent. K_M reflects the value of the equilibrium constant under the conditions of a hypothetical state where water acts only as a reactant not as a solvent, i.e., as a hypothetically "infinitely" small amount of water in pure methanol solvent. As the amount of water is

decreased, the value of K_{mix} increases, eq 4-3 and Figure 4-3 and 4-4, and the two terms in the product K_{mix} x $[D_2O]$ change in opposite directions. There are approximately compensating changes in the terms of this product and hence in the value of the ratio [AK]/[Im]. The compensation is not exact because the concentration (activity) of water changes more than the value of K_{mix}. The range in the variation in K_{mix} is given by the ratio of the equilibrium constants for the pure solvents K_M/K_D, 7.6 for 4-1 and 6.7 for 4-3. As the water concentration is reduced the net result is a decrease in the ratio of ketone to imine.

The variation in some of the results given for 4-3 in methanol-water in Table 4-1 now becomes understandable. Variable and small amounts of water in the commercial methanol account for the changes. Because the value of K_{mix} is so much larger for 4-3 than for 4-1 in the relationship K_{mix} $[D_2O]$ = [AK]/[Im], similar variations in the amount of water impurity will lead to a relatively larger change in the product ratio for the former.

Quantitative comparisons of the bioactivity of the solvent sensitive and labile 4-1 and 4-3 relative to those for other cholinergic substrates must be made with great care until it is established which one of the two forms involved in the hydrolytic equilibrium binds, if indeed it is only one form that is active. The data in Table 4-1 show that while the percentages of iminium ion from 4-1.HBr in water and in methanol vary modestly, 43 vs 100% (a factor of 2.5), the

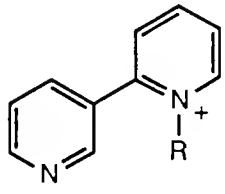
changes in the amount of ketone are much larger, 57% vs a trace. Therefore, estimates of the concentration of substrate undergoing binding based on the initial, total concentration uncorrected for the fractional amount of active material actually present may be grossly misleading. Similar errors may be made for 4-3.Cl, Table 4-1.

The composition of 4-1.HBr and 4-3.Cl in methanol-water is not unlike that for pure water but is higher in iminium ion, the amount depending on the quantity of water present. If methanol-water rather than pure methanol or pure DMSO is a reasonable approximation of the environment at a hydrophobic receptor binding site and there are no special electrostatic effects between substrate and binding site to perturb the equilibrium, then application of our earlier data [Chapter 2] about the composition of 4-1 in purely aqueous buffers should provide at least a semi-quantitative measure of concentrations in the hydrophobic medium.

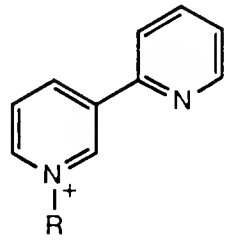
CHAPTER 5
PREPARATION OF 1-METHYL-2,3'BIPYRIDINIUM ION

Introduction

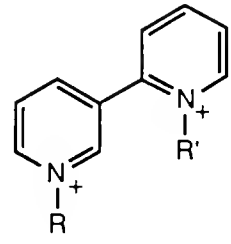
The 1-methyl-2,3'-bipyridinium ion (5-1) cannot be prepared by simple alkylation reactions. Treatment of 2,3'-bipyridine (BIPY) with methyl iodide or other alkylating agents gives selective quaternization at the sterically less hindered nitrogen of the 3-substituted ring to give products such as 5-2. BIPY is readily methylated at this position on reaction with methyl iodide at room temperature [75].



5-1, R = CH_3



5-2, R = CH_3

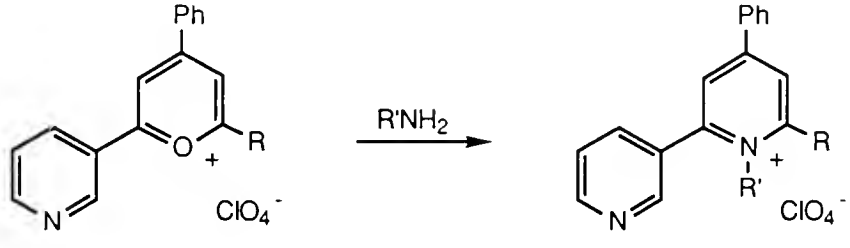


5-3, R = R' = CH_3

Once the first nitrogen of BIPY has been quaternized, the second more hindered nitrogen of the 2-pyridyl ring can be alkylated to form a dication. The second quaternization requires more forcing conditions than the first quaternization because of the steric hindrance due to the pyridyl group at the 2-position and because of the reduction of the nucleophilicity of the second nitrogen due to the

electron withdrawing effects of the pyridinium substituent. For example, the preparation of 1,1'-dimethyl-2,3'-bipyridinium diiodide (5-3,2I$^-$) requires heating a solution of BIPY and methyl iodide at 100 °C for a few hours [75].

An alternate synthetic pathway is required to prepare 1-alkyl substituted BIPY's. Substituted 1-alkyl BIPY's have been prepared by reactions of primary amines with pyrylium salts. By this method, 1-benzyl-2,4-diphenyl-2,3'-bipyridinium perchlorate (5-4) and 1-n-butyl-6-t-butyl-4-phenyl-2,3'-bipyridinium perchlorate (5-5) have been prepared from the corresponding pyrylium salts and amines (eq 5-1) [76]. The disadvantage of this type of synthesis is that the pyrylium salts must be prepared which may require several steps [77]. Some pyrylium salts can be dangerous to handle [77].



(5-1)

5-4, R = Phenyl, R'= Benzyl

5-5, R = t-Butyl, R' = n-Butyl

Another possible synthetic approach, the one adopted here, would be to block the more reactive nitrogen by a removable protecting group so that the less reactive nitrogen of the 2-pyridyl ring could then be alkylated. After this

selective alkylation, the protecting group could then be removed to give the 1-alkylated BIPY. This approach was taken to prepare 5-1, where R = CH_3.

Two different protecting groups were employed. One was the 2-(2-pyridyl)ethyl group. The 2-(2-pyridyl)ethyl and also the 2-(4-pyridyl)ethyl group have been used to protect heteroatoms in multistep syntheses. These groups have been used to protect NH groups in heterocycles, carboxylic acids, sulfides and sulfinic acids [78 - 80]. The quaternization of bipyridines with 2- and 4-vinylpyridine has been described. These procedures were reported to produce diquaternization of the bipyridines when 1 equivalent of the diprotonated bipyridine was reacted with an excess of the vinylpyridine [81].

The second protecting group used was the 2-(4-nitrophenyl)ethyl group. The kinetics and mechanisms of elimination reactions of N-(2-(4-nitrophenyl)ethyl)-alkylammonium ions have been studied [82, 83].

Both the 2-(2-pyridyl)ethyl and the 2-(4-nitrophenyl)-ethyl groups are similar in structure in that they consist of an ethyl group which is substituted in the 2-position by an electron withdrawing group. In one case, the electron withdrawing group is a 2-pyridyl ring and in the other it is a 4-nitrophenyl ring. Both of these electron withdrawing groups activate the α hydrogen of the ethyl group for elimination. The 2-(2-pyridyl)ethyl group is further activated for elimination by quaternization of the pyridine.

After alkylation of the more hindered nitrogen of BIPY, the protecting groups can be removed by treatment with base to promote the elimination reaction (Figure 5-1). This synthetic methodology also may be useful for selective alkylation of other heterocycles which contain more than one reactive center.

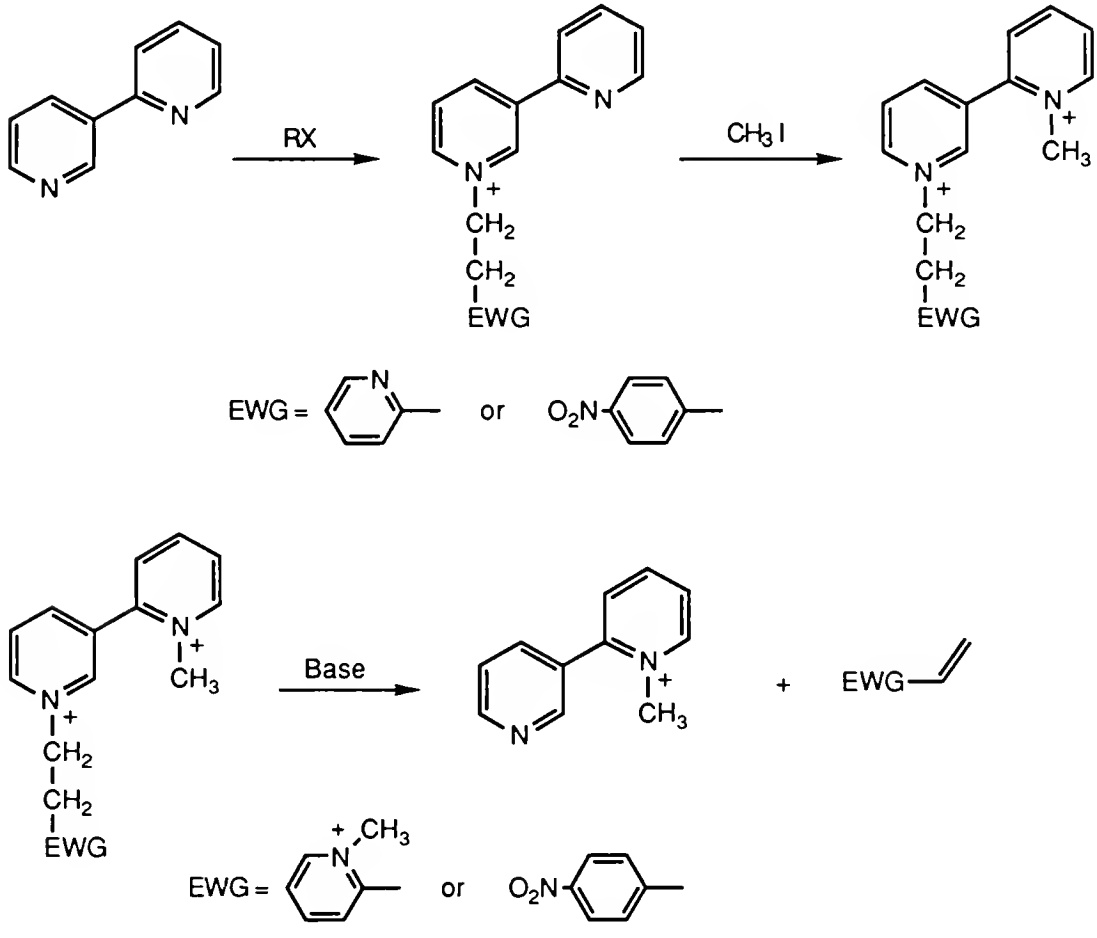


Figure 5-1. Scheme for selective alkylation of 2,3'-bipyridine using a removable protecting group.

Elimination from the pyridylethyl protected bipyridinium ion occurred under milder conditions than elimination from

the nitrophenylethyl protected bipyridinium ion. In addition, the nitrophenylethyl protected bipyridinium ion apparently forms a Meisenheimer-type σ complex on reaction with strong nucleophilic bases. Complex formation results in a slower formation of elimination products.

The 1-methyl-2,3'-bipyridinium cation is a biologically interesting molecule because of its structural similarities to the biologically active monoprotonated form of nicotine [53] and to the monoprotonated cyclic imine form of anabaseine. The reduced dihydro derivative of 5-1 is also of biological interest. While 5-1 will not readily permeate biological membranes because of its charge, the neutral dihydro derivative of 5-1 should be able to penetrate membranes. Once this dihydro derivative is within a cell, it can then be reoxidized by amine oxidases to form the bipyridinium cation. Reduction of 5-1 with borohydride should give the dihydro derivative [84].

For comparison, the 1'-methyl, the 1,1'-dimethyl, and mono- and diprotonated salts of 2,3'-bipyridine were prepared. The ^{1}H NMR spectra of these compounds proved interesting.

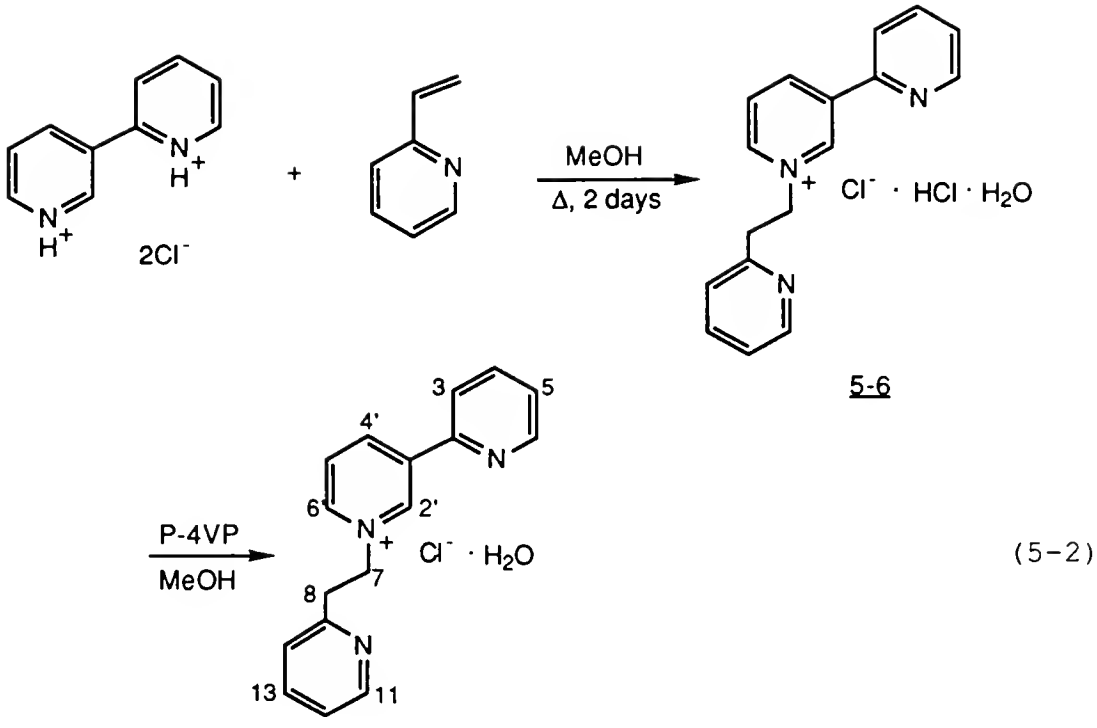
Results

Syntheses

The 2-(2-pyridyl)ethyl protecting group

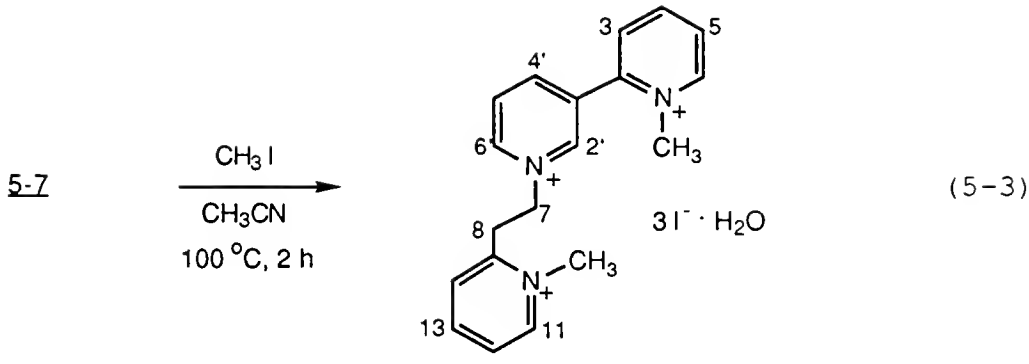
Heating a methanolic solution of 2,3'-bipyridinium dihydrochloride and 2-vinylpyridine at reflux for 2 days resulted in alkylation of the nitrogen of the 3-pyridyl ring through a Michael type addition. Presumably, an acid/base equilibrium is established between the 2,3'-bipyridinium dihydrochloride and the 2-vinylpyridine, activating both substances. Protonation of the nitrogen of 2-vinylpyridine makes it a better Michael acceptor; deprotonation of BIPY.2HCl provides a more reactive nucleophile. The product 5-6 was then isolated in 72% yield as the chloride hydrochloride salt which had to be deprotonated before it could be N-methylated. The free base 5-7 was prepared convienently in 75% yield from 5-6 by stirring a solution of 5-6 in methanol with a suspension of poly-4-vinylpyridine (P-4VP) resin (eq 5-2).

Heating a dried solution of 5-7 in acetonitrile with methyl iodide in a sealed tube at 100 °C for 2 hrs resulted in dimethylation of 5-7 to produce 5-8 in 24% yield. Both 2-pyridyl rings of the bipyridine and of the protecting group are methylated (eq 5-3). Methylation of the pyridylethyl protecting group makes it more electron



(5-2)

withdrawing and further activates the molecule for elimination while methylation of the bipyridine portion of the molecule makes it a better leaving group. Unfortunately, once the pyridylethyl group is methylated the molecule is so activated that it readily undergoes elimination under the reaction conditions and the nitrogen atom of the 3-pyridyl ring is no longer blocked. The reaction also yielded 1-methyl-2-vinylpyridinium iodide, 1'-methyl-2,3'-bipyridinium iodide, and an incompletely methylated product where only the pyridylethyl group is methylated. Methylation at lower temperatures resulted in incomplete methylation of 5-7 giving predominantly monomethyl material where the pyridylethyl group is methylated.



(5-3)

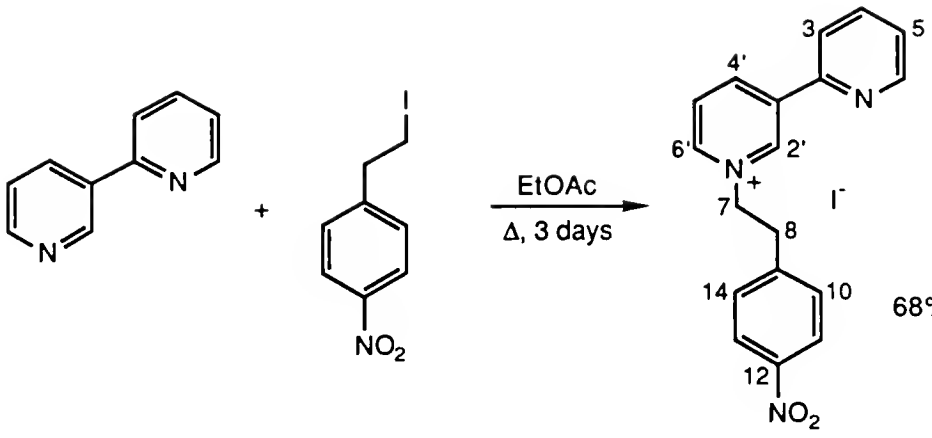
5-8

Because 5-8 was not obtained in high yield, the elimination reaction to produce 5-1 was never done on a preparative scale. Several microscale experiments showed that the protecting group could easily be removed. Bases as mild as poly-4-vinylpyridine resin and sodium bicarbonate promoted the elimination.

The 2-(4-nitrophenyl)ethyl protecting group

4-(2-Bromoethyl)-1-nitrobenzene was converted to the more reactive iodide before alkylation of the 3-pyridyl ring of BIPY. A solution of this iodide and BIPY in ethyl acetate was heated at reflux for 3 days to give 5-9 as a yellow precipitate in 68% yield (eq 5-4). The methylated dication, 5-10 was prepared in 79% yield by heating a solution of 5-9 and methyl iodide in acetonitrile in a sealed tube at 100 °C for 6 1/2 hrs (eq 5-5).

The nitrophenylethyl protecting group proved to be much more stable than the pyridylethyl group. In fact, elimination on a preparative scale proved to be difficult.



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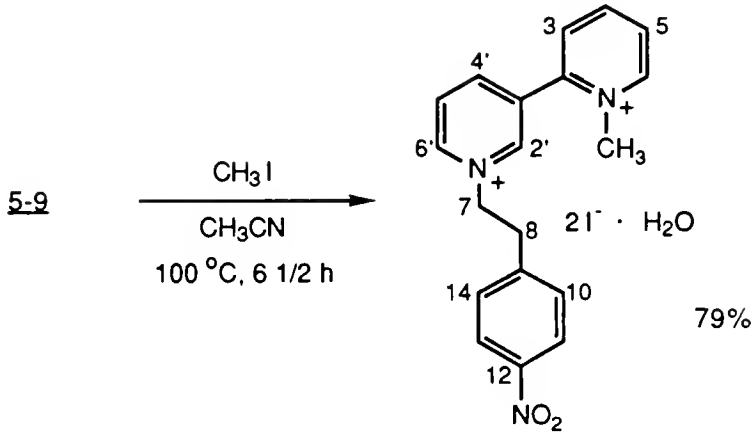
5-9

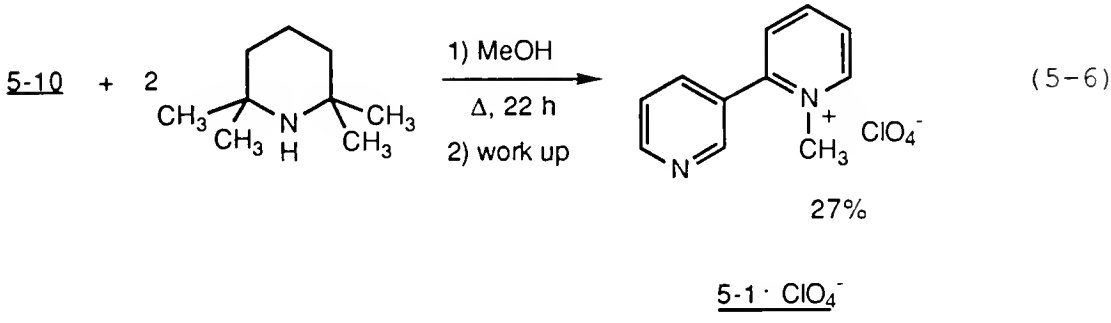


(5-5)

5-10

Elimination from 5-10 required a stronger base than that required for the elimination from 5-8. Mild bases such as pyridine or 4-dimethylaminopyridine would not induce elimination. Vigorous conditions produced by heating a solution of 5-10 in nitromethane with sodium carbonate resulted in loss of the methyl group to give 5-9 as the major product. Reaction of a solution of 5-10 in 1/1 DMF/acetonitrile with quinuclidine as a base also gave demethylation. Use of methoxide as a base gave elimination

products in a very slow reaction. When methoxide, a sterically unhindered nucleophilic base, was used to promote elimination, one or more σ adducts were formed as discussed below. Formation of these adducts slowed down the elimination reaction. Elimination from 5-10 was achieved successfully by using 2,2,6,6-tetramethylpiperidine, a sterically hindered base incapable of forming σ complexes with 5-10. Heating a suspension of 5-10 with a solution of 2,2,6,6-tetramethylpiperidine in methanol at reflux for 22 hrs afforded 5-1·ClO_4 in 27% overall yield following work up and conversion of the iodide to the perchlorate (eq 5-6).



(5-6)

Meisenheimer-type σ Adducts of 2,3'-Bipyridinium Dications

UV evidence for adducts

A solution of dication 5-10 in methanol gave peaks at 269 and 215 nm. When 2,2,6,6-tetramethylpiperidine was added, the solution turned yellow and dramatic changes in the UV spectrum occurred. Two peaks of similar intensity were present at 395 nm and at 260 nm with a shoulder at 280 nm.

In methanolic solution, an acid/base equilibrium is established between the piperidine base and solvent to produce methoxide and the protonated piperidinium ion. The methoxide that is produced most likely acts as the nucleophile and adds to the pyridinium rings of 5-10 to form σ adducts. No further change occurred in the spectrum after the solution stood for 40 min at room temperature. After standing 19 hrs at room temperature, the peak at 395 nm decreased in intensity relative to that at shorter wavelength. The short wavelength peak changed in appearance with a new maximum at 273 nm and a shoulder at 260 nm. These latter changes are most likely due to the slow elimination reaction which produces 4-nitrostyrene and 5-1. Addition of DABCO·$HClO_4$ resulted in the disappearance of color from the solution, the disappearance of the peak at 395 nm and the reappearance of a strong peak at 267 nm. DABCO (pKa 9.20 [46]) is a weaker base than the tetramethylpiperidine (pKa 11.10 [85]). Addition of DABCO·$HClO_4$ makes the solution less basic. The elimination reaction was not complete after 19 hrs and acidifying the solution apparently reverses the concurrent addition reaction and regenerates the dication, 5-10. Thus, an irreversible reaction such as that caused by nucleophilic attack by hydroxide and subsequent ring opening is not responsible for the observed UV changes on addition of base. Hydroxide ion might be formed reversibly from base and water released from the starting material, a hydrate or water impurity in the solvent.

In contrast, monocation 5-9 does not appear to form a methoxide adduct under similar conditions. A solution of 5-9 in methanol gave a UV spectrum consisting of a peak at 272 nm with a shoulder at 252 nm. Addition of 2,2,6,6-tetramethylpiperidine did not change the spectrum. The monoquaternized bipyridine 5-9 is not as electron deficient as the diquaternized bipyridine 5-10 and thus less susceptable to addition of nucleophiles.

1,1'-Dimethyl-2,3'-bipyridinium diidodide (5-3.2I^-) serves as a useful model for studying σ adducts of the 2,3'-bipyridinium dication because it cannot undergo elimination. A solution of 5-3.2I^- in methanol gave a stong narrow peak at 267 nm and a very small broad peak at 390 nm. Addition of 2,2,6,6-tetramethylpiperidine to the sample gave spectral changes similar to those observed on the addition of the piperidine to 5-10. The most intense peak was broad and appeared at 395 nm. Two other peaks were present at 287 and 255 nm. When DABCO·$HClO_4$ was added to the sample, the peak at 395 nm decreased in intensity and the intense peak at 268 nm reappeared. Hence, the change in the UV spectrum on addition of the piperidine and subsequent regeneration on addition of acid again provides evidence for the formation of a methoxide adduct of the bipyridinium dication. Moreover, the change in the spectrum on addition of the acid shows that 5-3 has not undergone an irreversible reaction.

NMR evidence for adducts

The methoxide induced elimination from 5-10 was followed by ^{1}H NMR in perdeuteromethanol containing one equivalent of methoxide. In a spectrum recorded 15 min after mixing, the signals for the pyridine protons and for the methylene protons α to the pyridine nitrogen (H7) appeared as broad humps and were shifted upfield. The protons of the nitrophenyl ring (H10 and H11) were relatively sharp and shifted insignificantly upfield. The N-methyl and H8 protons go from 4.30 and 3.64 ppm to 4.15 and 3.25 ppm, respectively, and are sharp. These shift changes are consistent with nucleophilic addition of methoxide to a pyridinium ring. Nucleophiles generally add to such heterocyclic cations at positions α and γ to the site of quaternization. Equilibration of the different possible methoxide adducts with each other and with the dication starting material on the NMR time scale gives rise to broad peaks. Because the addition is to the bipyridine portion of 5-10, the chemical shifts of the protons on the nitrophenyl ring are unaffected.

After 25 hours, the reaction mixture contained nearly equal amounts of starting material and elimination products based on the relative heights of the signals due to the protons meta to the nitro group (H10 and H14) in the adduct/dication mixture and in the nitrostyrene product which occur at 7.59 and 7.68 ppm, respectively. The elimination was complete after 4 days. Deuterium exchange was observed at

the positions α to the quaternized nitrogen atoms. This exchange takes place through base catalyzed ylid formation [86].

Another interesting result is obtained from this experiment. No deuterium exchange is observed in the vinyl proton α to the nitrophenyl ring in the nitrostyrene product as indicated by an absence of smaller coupling to the β protons associated with deuterium. This result suggests that the elimination occurs either through an E2 or $E1cb_{irr}$ and not by an E1cb mechanism.

Another NMR experiment was done under conditions similar to those used for the preparative scale elimination. An NMR sample which contained 5-10 and 1.2 equivalents of 2,2,6,6-tetramethylpiperidine dissolved in CD_3OD was heated at 56 °C to effect the elimination and ^1H NMR spectra were recorded at room temperature after several time intervals. Again, the spectrum broadened after the addition of base. The elimination reaction proceeded slowly; unreacted starting material was still present after heating for 45 hrs. The piperidine apparently deprotonates the methanol to generate methoxide ion which acts as the nucleophile in adduct formation.

Adducts were also observed by NMR in solutions of 5-10 in DMSO-d_6 when 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used as a base. Again, the water of hydration from 5-10 is apparently deprotonated by the DBU to form hydroxide which is responsible for formation of σ complexes. In DMSO, the

signals for the adducts and starting material are sharp but the spectra are very complex. The spectra contained signals due to a mixture of starting material, elimination products, adducts, and DBU.

Because of the complexity of the NMR spectra of 5-10 under conditions where adducts are formed, 5-3 was used as a model. Two equivalents of 2,2,6,6-tetramethylpiperidine were added to an NMR sample which contained 5-3.2I$^-$ dissolved in a solution of 4/1 DMSO-d_6/methanol-d_4. In a spectrum recorded 15 min after the addition of the piperidine, the sample contained a 3/1 mixture of dication 5-3 and a single σ adduct (5-11). Again, an acid/base equilibrium is established between the piperidine and methanol solvent to produce methoxide which most likely acts as the nucleophile (Figure 5-2). No change in the spectrum was observed 1 3/4 hrs after the addition of the piperidine. When a proton spectrum was recorded 10 min after the addition of 2 equivalents of DCl, only 5-3 was present. Therefore, the formation of the sigma complex is fast and reversible.

The chemical shifts and coupling constants of the protons of the adduct indicate unequivocally that methoxide addition occurs at the 6-position of the 3-pyridyl ring. The formation of Meisenheimer-type σ complexes results in upfield shifts of pyridine signals with the proton at the site of addition showing the largest shift [87, 88]. In this case, H6' shows the largest upfield shift. All of the proton signals of the adduct are shifted upfield relative to those

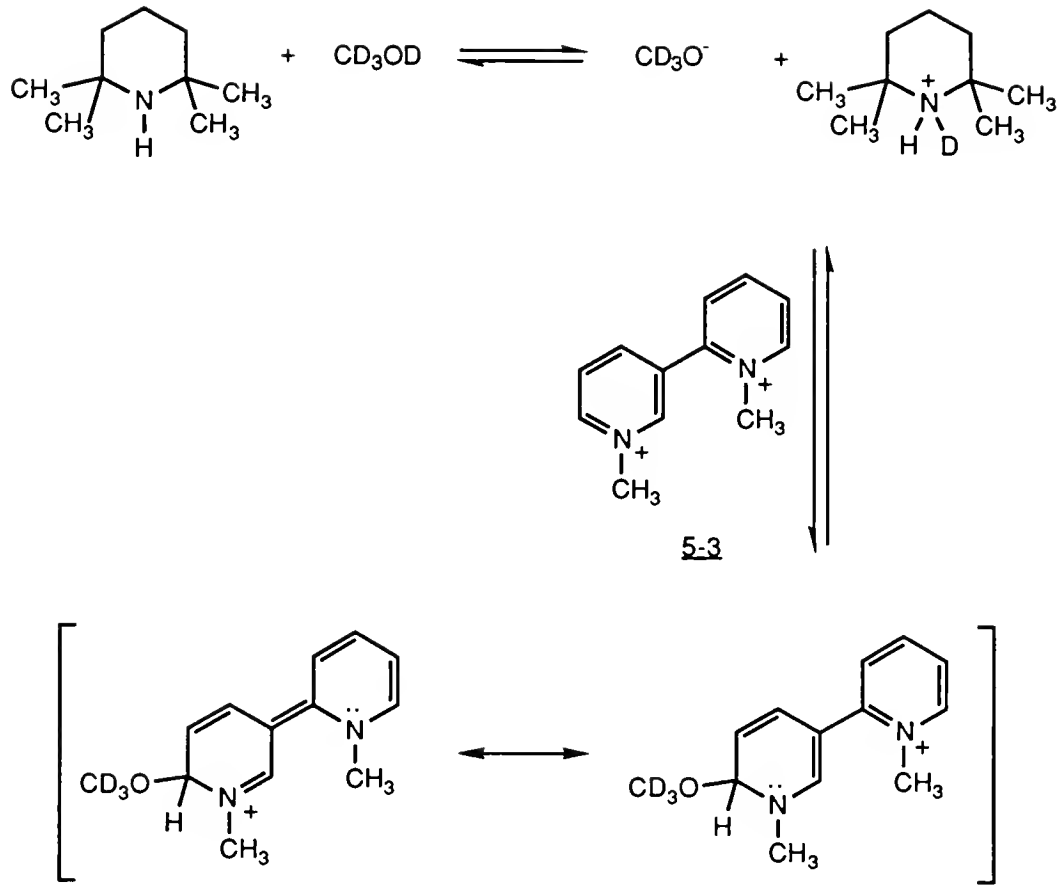


Figure 5-2. Formation of a methoxide adduct of 1,1'-dimethyl-2,3'-bipyridinium ion in methanol-d_4.

of the diquaternized bipyridine and to those of a simple pyridinium ion (Tables 5-1 and 5-2) because adduct formation increases the electron density of both rings as expected since cross-conjugation between the rings is possible. If the nucleophile had added to the 2-pyridyl ring, no delocalization into the other ring would be possible and the chemical shifts of the 3-pyridyl ring should be like those of a 3-substituted pyridinium ion. Only addition to the

Table 5-1. Comparison of Chemical Shifts and Coupling Constants for ^1H NMR Spectra of 5-3 and 5-11 in 4/1 DMSO-d_6/CD_3OD relative to TSP.

	5-3		5-11	
Proton	Chemical Shift / ppm[a]	Coupling Constants / Hz	Chemical Shifts / ppm[a]	Coupling Constants / Hz
H2'	9.502	s	7.678	s
H4'	9.010	d, $J_{4,5}$=8.1	6.876	d, $J_{4,5}$=9.8
H5'	8.472[b]/8.30-8.43[c]	unresolved dd / m	5.455	dd, $J_{4,5}$=9.8 $J_{5,6}$=4.5
H6'	9.364/9.324[d]	d, $J_{5,6}$=6.2 / d, $J_{5,6}$=6.1	5.744	d, $J_{5,6}$=4.5
H3	8.30-8.43[c]	m	7.948	d, $J_{3,4}$=8.2
H4	8.869	t, $J_{3,4}$=$J_{4,5}$=7.8	7.738	t, $J_{3,4}$≈$J_{4,5}$=6.9[e]
H5	8.472[b]/8.30-8.43[c]	unresolved dd / m	8.30-8.43	m
H6	9.364/9.324[d]	d, $J_{5,6}$=6.2 / d, $J_{5,6}$=6.1	8.800	d, $J_{5,6}$=6.1
1'-NCH_3	4.53	s	3.318	s
1-NCH_3	4.326	s	4.216	s

[a]TSP was used as an internal reference.
[b]Cannot distinguish between H5 and H5' based on coupling constants.
[c]Multiplet containing 3 protons.
[d]Cannot distinguish between H6 and H6' based on coupling constants.
[e]Unsymmetrical triplet with J=7.1 and 6.6 Hz.

Table 5-2. Chemical Shift Differences Between Protons of 5-3 and 5-11 in 4/1 DMSO-d_6/CD_3OD.

Proton	Chemical Shift Difference / ppm[a,b]
H2'	-1.82
H4'	-2.13
H5'	-3.02/-2.9[c]
H6'	-3.62/-3.58[d]
H3	-0.42
H4	-1.13
H5	-0.107/0[c]
H6	-0.56/-0.52[d]
1'-NCH_3	-1.21
1-NCH_3	-0.11

[a]Negative shift differences represent an upfield shift on formation of 5-11.
[b]TSP was used as an internal reference.
[c]Cannot distinguish between H5 and H5' in 5-3 based on coupling constants.
[d]Cannot distinguish between H6 and H6' in 5-3 based on coupling constants.

3-pyridyl ring allows conjugation of the two rings. The peaks assigned to H4', H5', and H6' are shifted farther upfield than those assigned to the protons of the 2-pyridyl ring, indicating they are on the ring containing the added nucleophile. The 4, 5, and 6 protons of the 3-pyridyl ring form a coupled 3 spin system whereas the 3, 4, 5, and 6 protons on the 2-pyridyl ring form a 4 spin system. The coupling pattern of the peaks assigned to H4' (d, $J_{4,5}$ = 9.8 Hz), H5' (dd, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 4.5 Hz), and H6'

(d, $J_{5,6}$ = 4.5 Hz) is consistent with the pattern expected for a 3 spin system. A four spin system would be expected to give a coupling pattern consisting of two doublets and two doublets of doublets or two triplets.

Deuterium is incorporated into both the adduct and the dication, a significant and revealing observation. In the spectrum recorded 1 3/4 hrs after addition of the piperidine, 73% deuteriation was observed at the 2'-position of 5-3 and 68% deuteriation was found at the 2'-position of 5-11. Spectra were not recorded with appropriate delay times between transients to allow accurate quantitation of peaks so that the amount of deuterium incorporation in both molecules is likely to be the same within experimental error.

Deuterium exchange in pyridinium ions takes place through base catalyzed ylid formation [86]. An electron withdrawing substituent at the 3-position of a pyridinium ion makes the 2-position more activated for isotope exchange than the 4- or 6-positions [89]. Because the charge on nitrogen is neutralized on formation of the methoxide adduct, the adduct is expected to undergo deuterium exchange much more slowly than the dication.

The observation that both species undergo deuterium exchange to the same extent indicates that the starting material and adduct are in rapid equilibrium. This is an important conclusion because it suggests that adduct formation during attempted deprotection, although reversible,

nevertheless gives rise to a reduced rate of elimination. Adduct with its poorer leaving group is less reactive.

^{1}H NMR of N-Methylated 2,3'-Bipyridines and Protonated 2,3'-Bipyridines

There are some unique and informative changes in the chemical shifts of the different N-methylated 2,3'-bipyridinium ions in comparison with the protonated 2,3'-bipyridines. In the 1'-methylated BIPY (5-2), the protons of the 3-pyridyl ring are more deshielded than those of the 2-pyridyl ring due to quaternization of the 1' nitrogen. The N-methyl peak falls at 4.527 ppm (Table 5-3). Interestingly, when the second nitrogen is quaternized to form 5-3, the chemical shifts for H2' and H4' move upfield from 9.374 to 9.298 ppm and 9.011 to 8.890 ppm, respectively, while all the other peaks move downfield. The second N-methyl peak falls at 4.289 ppm which is similar to the shift for the methyl group of 1-methyl BIPY which falls at 4.248 ppm. In contrast, the chemical shifts for all of the protons of the diprotonated BIPY move downfield, including H2' which goes from 9.234 to 9.395 ppm and H4' which goes from 8.933 to 9.098 ppm on going from the monohydroperchlorate to the dihydrochloride salt.

Table 5-3. Chemical Shifts of Protonated and N-Methylated 2,3'-Bipyridinium Ions in D_2O.

Proton	BIPY[a]	5-12[b]	5-13[b]	5-2[b]	5-3[b]	5-1[b]
H2'	9.199	9.234	9.395	9.374	9.298	8.784
H4'	8.322	8.933	9.098	9.011	8.890	8.04-8.19[c]
H5'	7.406	8.10-8.19[d]	8.30-8.37[d] /8.060[e]	8.197	8.387/8.274[e]	7.767
H6'	8.655	8.849	9.044/8.934[f]	8.878	9.167/9.099[f]	8.843
H3	7.72-7.83[d]	8.042	8.30-8.37[d]	8.050	8.213	8.04-8.19[c]
H4	7.72-7.83[d]	8.10-8.19[d]	8.602	8.112	8.754	8.655
H5	7.291	7.666	8.30-8.37[d] /8.060[e]	7.641	8.387/8.274[e]	8.04-8.19[c]
H6	8.726	8.732	9.044/8.934[f]	8.761	9.167/9.099[f]	8.964
1'-NCH_3	--	--	--	4.527	4.565	--
1-NCH_3	--	--	--	--	4.289	4.248

[a]In $CDCl_3$ with TMS as an internal reference.
[b]TSP was used as an internal standard.
[c]Multiplet containing 3 protons.
[d]Multiplet containing 2 protons.
[e]Cannot distinguish between H5 and H5' based on coupling constants.
[f]Cannot distinguish between H6 and H6' based on coupling constants.

Discussion

Syntheses

The 1-methylated 2,3'-bipyridium cation has not been made previously. Simple alkylation reactions alkylate the nitrogen of the sterically less hindered 3-pyridyl ring selectively. Although the methods of preparation of 1-methyl-2,3'-bipyridinium ion described in this work do not give high overall yields, their advantage lies in the fact that they consist of only a few very simple steps: (1) alkylation to protect the nitrogen of the 3-pyridyl ring from unwanted reaction, (2) methylation of the hindered nitrogen and (3) deprotection of the 3-pyridyl ring. The first two steps proceed in good yield but the final elimination reaction of 5-10 was not optimized. Higher yields may be obtained by the use of a dry aprotic solvent which will not react with the base to form a nucleophile that can add to a ring. Moreover, water from the hydrated starting material may produce hydroxide ion on reaction with base which in turn can react with the dication to give products where the pyridinium ring is opened [90].

Both 5-8 and 5-10 are activated towards elimination by the electron withdrawing 1-methylpyridinio and 4-nitrophenyl rings, respectively. The bipyridine portion of the molecule makes a good leaving group and methylation of the 2-pyridyl

ring makes it an even better leaving group. The two pKa's of 2,3'-bipyridine are 4.4 and 1.5 [29]. Only mild bases such as poly-4-vinylpyridine resin and sodium bicarbonate are required to promote the elimination reaction of 5-8 while the elimination reaction of 5-10 requires a much stronger base. Use of a strong nucleophilic base which promotes the elimination reaction of 5-10 also results in the formation of σ adducts where the nucleophile adds to the electron deficient bipyridinium rings. The adduct would only be expected to undergo elimination very slowly if at all. In addition, formation of these adducts lowers the concentration of both free 5-10 and base in solution and reduces the rate of elimination. Therefore, addition of methoxide ion to the mixture may be without much benefit as it converts starting material into less reactive adduct and for those cases where adduct formation is extensive an increase in methoxide concentration is exactly counterbalanced by a corresponding decrease in substrate concentration owing to adduct formation. It is understandable that in the NMR experiment where methoxide was used as a base in methanol solvent, the elimination reaction took a few days to reach completion.

Formation of Meisenheimer-Type σ Adducts of Diquaternized 2,3'-Bipyridinium Ions

Pyridinium ions which are substituted with electron withdrawing substituents form Meisenheimer-type σ adducts more readily than unsubstituted pyridinium ions.

The electron withdrawing groups destabilize the electron deficient pyridinium ion relative to the neutral adduct [88, 90]. Cations such as quinolinium and benzoquinolinium ions which contain fused benzene rings also form adducts more readily than simple pyridinium ions [90]. The loss in resonance energy on going from cation to adduct is not as great in systems with fused aromatic rings as for the unsubstituted pyridinium ion.

Dialkylation of BIPY makes it sufficiently electron deficient that it forms σ adducts readily. In principle, the formation of six different methoxide adducts of 5-3 or 5-10 is possible where the nucleophile adds to the positions α and γ to both quaternized nitrogens. Hard nucleophiles generally react at the 2- and 6-positions while soft nucleophiles react at the 4-position. Methoxide and ethoxide have been shown to react rapidly at the 2-position of 1-phenyl substituted pyridinium ions to form σ complexes [88]. Thus, methoxide would also be expected to attack the 2- and 6-positions of 5-3 and 5-10. Addition of methoxide at the 2- and 4-positions of the 3-pyridyl ring would be expected to be less favorable than addition at other sites because of the steric hindrance due to the pyridyl ring attached at the 3-position. Similarly, addition of methoxide to the 2-position of the 2-pyridyl ring would also be expected to be less favorable because of steric hindrance. Nucleophiles such as methoxide ion were found to add to the unsubstituted positions of 1,2,4-triphenylpyridinium ion and 1,2,6-triphenylpyridinium

ion regardless of the hard/soft character of the nucleophile [87]. Of the 3 remaining sites for addition, only addition of the nucleophile to the 6-position of the 3-pyridyl ring will allow delocalization of the electrons into the other ring. This delocalization of electrons provides more extended conjugation which helps compensate for the loss of aromaticity on forming the σ adduct. In addition, the delocalization reduces the charge in both rings which further stabilizes the adduct relative to the dication. The observed adduct therefore is likely to be the thermodynamic product.

^{1}H NMR of N-Methylated 2,3'-Bipyridines and Protonated 2,3'-Bipyridines

The changes in the ^{1}H NMR spectra of the N-methylated BIPY's, suggest that there are conformational changes in BIPY on quaternization of the nitrogen of the 2-pyridyl ring. Usually quaternization causes a downfield shift in the signals of the pyridine protons due to the electron withdrawing effect of the charged nitrogen. In a bipyridinium ion, this effect is greatest in the ring where the nitrogen is quaternized. The protons of the unquaternized ring of a bipyridinium ion also show downfield shifts as the overall electron withdrawing character of the attached ring is increased on quaternization. These trends are also expected on protonation of the ring nitrogens and our observations for protonation agree with these

expectations. The chemical shifts for the diprotonated BIPY are downfield of those for both the monoprotonated BIPY and the monomethylated BIPY. However, on going from the monomethylated BIPY (5-2) to the dimethylated BIPY (5-3), downfield shifts are observed for protons H6' and H5' as expected but apparently anomolous upfield shifts are observed for the H2' and H4' protons. Consideration of other compound provides additional insight.

In 2,2'-bipyridine, the H3 and H3' protons are deshielded relative to H5 and H5' which are both β to the ring nitrogens. This desheilding is due both to the electrostatic field effect of the nitrogen lone pair on the neighboring ring and to the magnetic anisotropy of the neighboring ring. The effect of the dipole of the nitrogen lone pairs on the chemical shifts of H3 and H3' is reduced in polar hydrogen bonding solvents such as D_2O so that the major contribution to the deshielding is from the anisotropy of the neighboring ring [91]. In bridged diquaternary 2,2'-bipyridinium ions, the chemical shift of the H3 proton moves upfield as the angle between the planes of the rings is increased by increasing the length of the bridge. The difference between the chemical shifts of H5 and H3 can be used as a measure of the angle between the planes of the 2 rings [92]. The observation that the signals for H2' and H4' move upfield on going from 5-2 to 5-3 suggests that the average angle between the planes of the rings increases on quaternization. The methyl group on the 2-pyridyl ring of 5-

3 exerts a steric effect on the molecule so that a coplanar conformation of the rings is less favorable than in 5-2.

In bridged o,o-dimethylbiphenyls and bridged diquaternary 3,3'-dimethyl-2,2'-bipyridinium ions, the chemical shift of the methyl protons was found to move upfield as the length of the bridging unit and the angle between the planes of the two rings are increased [93, 94]. The methyl groups move into the shielding region of the neighboring aromatic ring as the angle between the planes of the two rings is increased. This type of shielding effect seems to be present in 5-1 and 5-3. The 1'-methyl of 5-2 falls at 4.527 ppm which is similar to the shift of the N-methyl of another bipyridinium ion, 1-methyl-4,4'-bipyridinium ion, which falls at 4.464 ppm. But the 1-methyl of 5-1 falls much higher at 4.248 ppm and is shielded relative to these methyls. This shielding effect can again be explained by a conformation which places the methyl group in the shielding region of the 3-pyridyl ring. The preferred conformation of 5-1 therefore is likely to be non-planar.

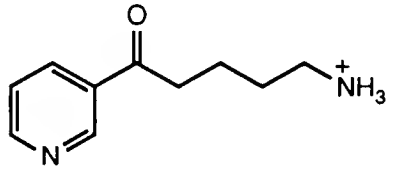
CHAPTER 6
SYNTHESIS OF ANABASEINE, ANABASEINE ANALOGUES, AND BIPYRIDINES

Introduction

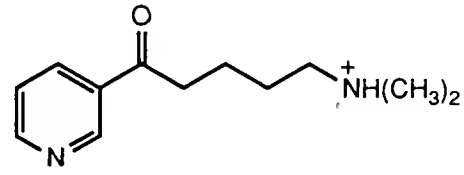
Because of the activity of anabaseine, a new synthesis was desired to produce anabaseine in higher yields for biological testing. Yields obtained routinely from the classical preparation [59] were only about 4% [95]. Anabaseine and other imines which have aryl substituents on the imine carbon can be synthesized a by Claisen-type condensation of an aryl ester with a lactam followed by hydrolysis and decarboxylation of the resulting β-ketoamides. Procedures have been reported for the syntheses of myosmine [96] and 5-fluoroanabaseine [97] which are based on Claisen-type condensations of nicotinic esters with 5- and 6-membered lactams. These procedures use LDA as a base and a trimethylsilyl protecting group for the amide nitrogen rather than sodium ethoxide base and benoyl protecting group of the classical synthesis [59].

An analogue of the open chain amino ketone form of anabaseine was prepared so that its activity could be compared with that of anabaseine to help assess which form of anabaseine is responsible for biological activity. 5-(N,N-Dimethylamino)-1-(3-pyridyl)-1-pentanone has a dimethylamino

group in place of the amino group of anabaseine so that it is unable to cyclize. At physiological pH, the dimethylamino group will be protonated like the amino group of anabaseine but the charge is not permanent and the neutral free base should be capable of penetrating biological membranes unlike a permanently charged trimethylammonio derivative.



Anabaseine



Dimethylamino Analogue of Anabaseine

(S)-N-methylanabasine represents an analogue of both nicotine and the cyclic iminium form of anabaseine with a structure consisting of a 1-methylpiperidine ring attached at its 2-position to the 3-position of a pyridine ring. A chiral center is present at the 2-position of the piperidine ring as in pyrrolidine ring of nicotine. The naturally occurring S-isomer of nicotine is more active than the R-isomer [98], therefore the (S)-isomer of N-methylanabasine is expected to be more active than the (R)-isomer. The monocationic form of nicotine where the pyrrolidine ring is protonated is believed to be active [53]. N-methylanabasine will also be protonated at physiological pH.[1] A comparison

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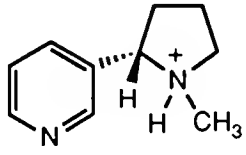
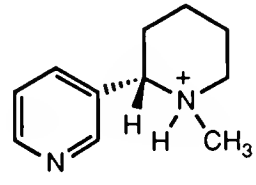
[1] The pKa's of the nitrogen atoms in the 5- and 6-membered rings are likely to be similar. The pKa's of 1,2-dimethylpyrrolidine and 1,2-dimethylpiperidine are the same, 10.26.[51]

of biological activities of N-methylanabasine and nicotine to see what effect of increasing the ring size will have on the activity should prove interesting.

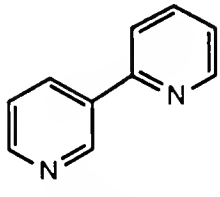


(S)-Nicotine Monocation

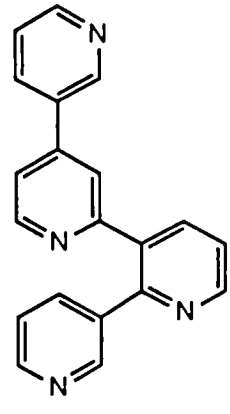


(S)-N-Methylanabasine Monocation

Two other alkaloids isolated from hoplonemertines are 2,3'-bipyridine and nemertelline, a tetrapyridyl [13]. A third alkaloid, not yet identified, has a mass spectrum consistent with a methyl substituted bipyridine [Kem, unpublished results]. A method of synthesizing substituted bipyridines and polypyridines was desired both for identification of natural products and for obtaining larger amounts for biological testing. Synthetic analogues with different substitution patterns were also desired to compare the effects of substituents on biological activity.



2,3'-Bipyridine



Nemertelline

Recently, a method was reported for palladium (0) catalyzed coupling of pyridylboranes with halopyridines to form bipyridines and terpyridines [17, 18]. This methodology was used by us to prepare substituted bipyridines.

Results and Discussion

Synthesis of Anabaseine and Cyclic Imine Analogues

Anabaseine was synthesized by LDA initiated condensation of N-protected 2-piperidone with ethyl nicotinate followed by hydrolysis and decarboxylation of the resulting β-ketoamide (Figure 6-1). N-protected 1-trimethylsilyl-2-piperidone was prepared *in situ* by treating 2-piperidone with LDA followed by trimethylsilyl chloride. Isolation of the protected piperidone as reported is not necessary [97]. Addition of a second equivalent of LDA to the protected piperidone followed by the nicotinic ester forms the amide enolate which condenses with the added ester. The trimethylsilyl protecting group is very labile and is removed by hydrolysis. The enolate of 3-nicotinoyl-2-piperidone (6-1) is water insoluble and can be isolated as a stable solid and used without further purification. This solid was never characterized and may consist of a mixture of salts including the lithium enolate, the deprotonated amide, lithium hydroxide and the neutral ketoamide.

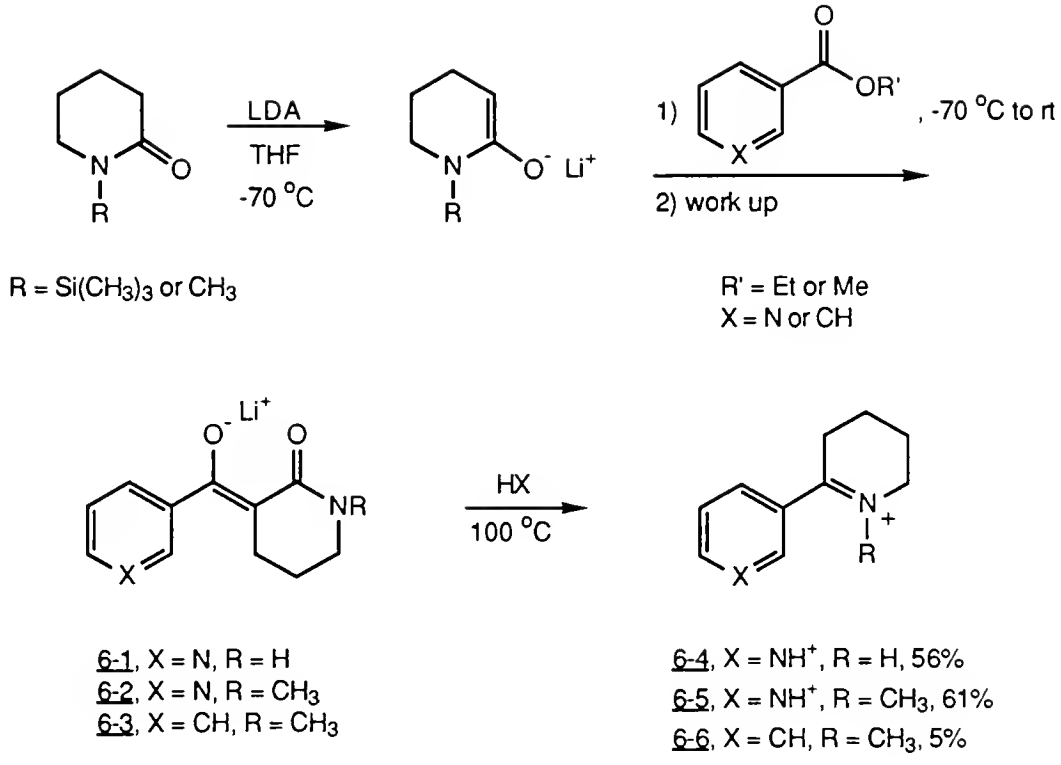
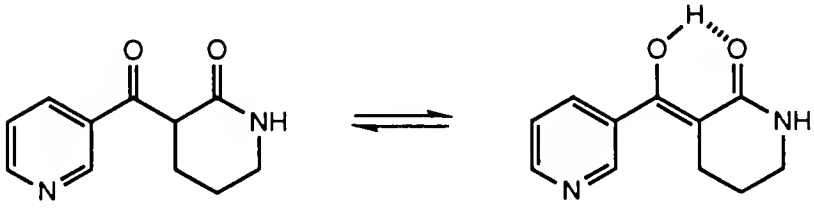


Figure 6-1. Synthesis of 2-aryl substituted 1-piperideines.

Two separate samples of 6-1 were neutralized by the addition of acid to an aqueous solution of the enolate. The first was extracted into ethyl acetate and yielded a white solid with a melting point of 114-122 °C. After extraction of the second sample into ethyl acetate and recrystallization once from ethyl acetate and once from ethanol a white solid was obtained with a melting point of 127-129 °C. Both samples gave elemental analyses expected for the neutral 3-nicotinoyl-2-piperidone (6-7).

3-Nicotinoyl-2-piperidone, a β-ketoamide, can tautomerize to an enol form (eq 6-1). The two solids most likely contain differing amounts of keto and enol forms of

6-7 which accounts for the difference in melting points. A proton NMR of the low melting solid in $CDCl_3$ recorded 45 min after preparing the sample showed a mixture of 58% enol and 42% keto tautomers. A proton NMR spectrum of the higher melting solid in $CDCl_3$ recorded 15 min after mixing contained almost exclusively enol tautomer. After 22 hrs, the ratio changed to 71% enol and 29% keto tautomers and after 2 days the sample contained 61% enol and 39% keto tautomers. The ratio did not change further on standing.



(6-1)

6-7

Anabaseine was formed on hydrolysis and decarboxylation of 6-1 in either refluxing concentrated aqueous HCl or HBr. The dihydrochloride (6-4.2HCl) can be induced to crystallize by diluting the reaction mixture with isopropyl alcohol. Analysis of this salt shows that an equivalent of water is present. It is not known whether the solid exists as the cyclic imine with water of hydration in the crystal lattice or as the hydrolyzed open chain amino ketone.

Isolation of the diprotonated salts rather than the neutral form of anabaseine results in improved yields. Anabaseine dihydrochloride was prepared in 56% yield from

ethyl nicotinate and 2-piperidone. At high pH in aqueous solution, anabaseine exists as the imine free base [Chapter 2]. Once isolated the free imine decomposes easily, making purification difficult and lowering the yields. The diprotonated salts are stable solids and can be purified easily by recrystallization.

N-Methylanabaseine and the 2-phenyl substituted 1-methylpiperideinium ion can be synthesized in the same way as anabaseine (Figure 6-1). Condensation of 1-methyl-2-piperidone with ethyl nicotinate produced the 1-methyl-3-nicotinoyl-2-piperidone enolate (6-2) which gave N-methylanabaseine chloride hydrochloride (6-5.Cl.HCl) on hydrolysis and decarboxylation in concentrated HCl in 61% yield. Analysis of this salt shows that it also contains an equivalent of water. Likewise, condensation of 1-methyl-2-piperidone with methylbenzoate gave the 1-methyl-3-benzoyl-2-piperidone enolate (6-3) which was also hydrolyzed and decarboxylated in concentrated acid to give the imine (6-6).

The pyridinium salts of anabaseine and N-methylanabaseine can be converted to the free bases by treating solutions in methanol with a suspension of poly-4-vinylpyridine. Thus 6-4.2HCl, 6-4.2HBr, and 6-5.Cl.HCl can be converted to 6-4.HCl, 6-4.HBr, and 6-5.Cl, respectively. These monocationic salts contain an equivalent of water as indicated by elemental analysis.

Other derivatives of anabaseine can be prepared which have imine rings of different size and different aryl

substituents using this method. Comparison of the effects of substitution and changes in ring size on the position of the hydrolysis equilibrium with biological activity will prove interesting.

Preparation of 5-(N,N-Dimethylamino)-1-(3-pyridyl)-1-pentanone

Claisen condensation of ethyl 5-(N,N-dimethylamino)pentanoate with ethyl nicotinate followed by hydrolysis and decarboxylation of the resulting β-ketoester afforded 5-(N,N-dimethylamino)-1-(3-pyridyl)-1-pentanone (6-10) (Figure 6-2). Ethyl 5-(N,N-dimethylamino)pentanoate (6-8) was prepared in 49% yield by alkylation of dimethylamine with ethyl 5-bromopentanoate in the presence of sodium iodide to convert the bromide to the more reactive iodide. In addition to the desired product, the diester resulting from dialkylation of dimethylamine was obtained in 17% yield based. Treatment of 6-8 with LDA followed by ethyl nicotinate gave ethyl 5-(N,N-dimethylamino)-2-nicotinoylpentanoate (6-9) on work up. The ketoester 6-9 was hydrolyzed and decarboxylated in concentrated HBr at 100 °C and 5-(N,N-dimethylammonio)-1-(3-pyridinio)-1-pentanone dihydrochloride (6-10.2HCl) was isolated in 29% yield following conversion of the bromide to the chloride.

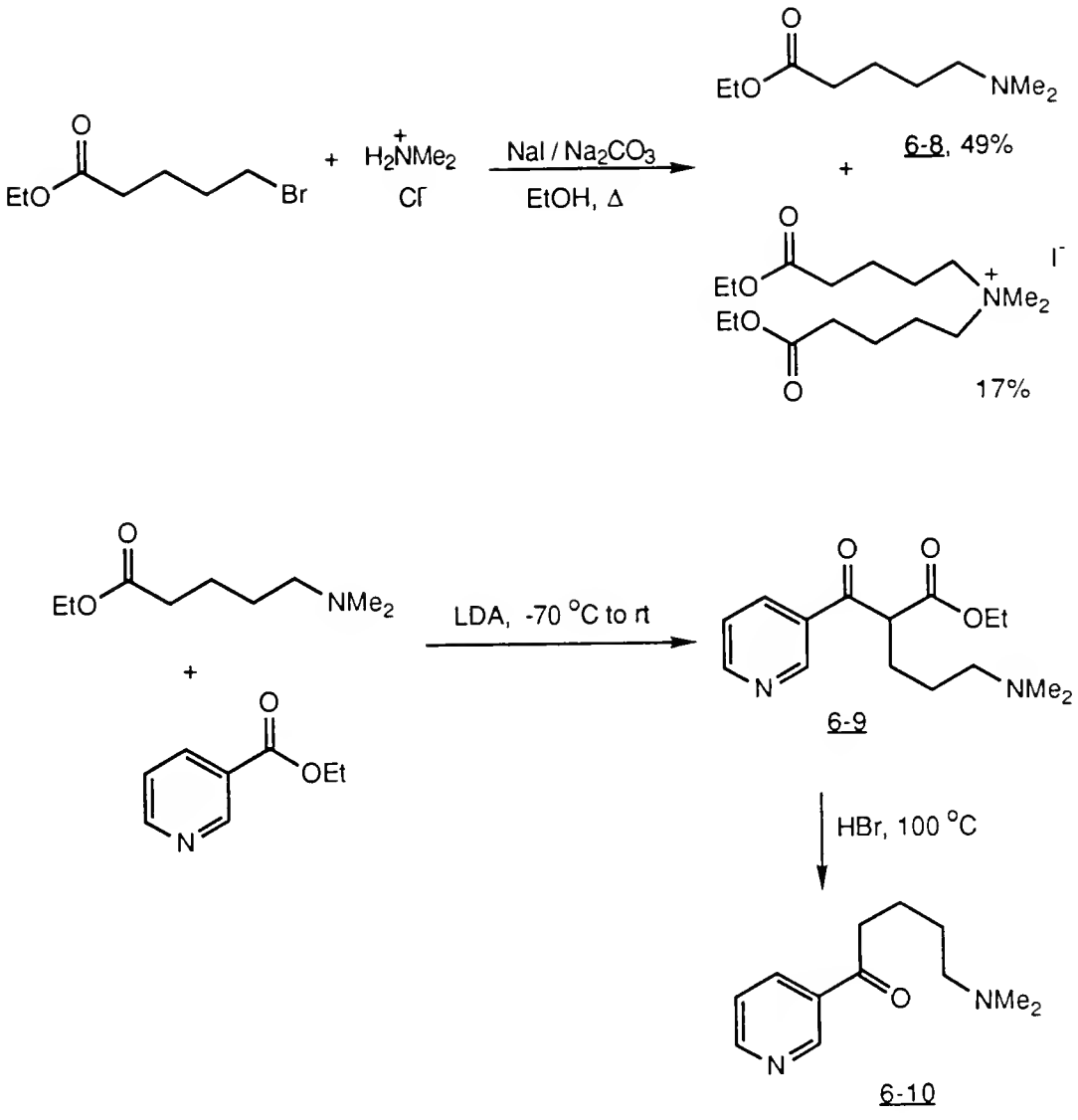
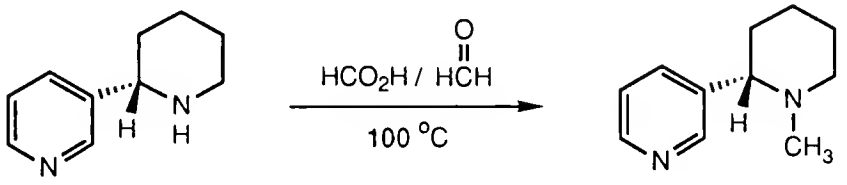


Figure 6-2. Synthesis of an open-chain analogue of anabaseine.

(S)-N-Methylanabasine

Optically active (S)-N-methylanabasine (6-11) was prepared as reported [99] by Eschweiller-Clarke reductive methylation of the optically active (S)-anabasine, a naturally occuring tobacco alkaloid. After a solution of

formaldehyde, formic acid, and anabasine was heated at 100 °C for several hours, N-methylanabasine was obtained as a yellow liquid (Equation 6-2).



(6-2)

6-11

Palladium Catalyzed Cross-Coupling of Heteroaromatic Molecules

Recently, many methods have been reported for palladium (0) catalyzed coupling of aryls to form biaryls and the coupling of alkenes to form dienes. Some of these methods involve coupling of aryl halides with heteroarylstannanes, heteroarylboranes, or heteroarylboronic acids. The mechanism for coupling of alkenyl triflates with alkenylstannanes has been investigated [100 - 102]. The reaction proceeds through oxidative addition of the alkenyltriflate to Pd(0) to form a Pd(II) complex followed by transmetallation of the alkenylstannane to give a new Pd(II) complex. The alkenes on the metal are then coupled in a reductive elimination to regenerate the Pd(0) which can be used catalytically.

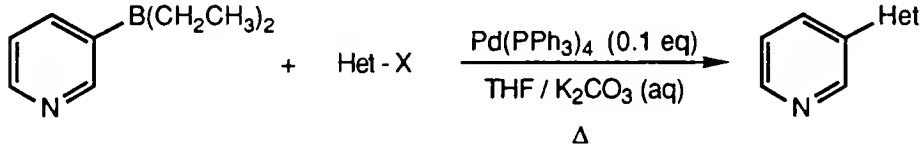
Coupling of pyridylboranes with heteroaryl halides, aryl halides and alkenyl halides has been used to prepare

heteroarylpyridines [17, 18], arylpyridines [15], and alkenylpyridines [16]. These coupling reactions presumably go through the same oxidative addition/reductive elimination mechanism as the coupling of alkenyl triflates with alkenylstannanes. An oxide base must be present in the reaction mixture to convert the borane to the reactive borate ion adduct.

Aryl bromides tend to be more reactive than aryl chlorides towards Pd(0) catalyzed coupling. For pyridyl halides, the reactivity is dependent on the position of substitution with the order of reactivity being 4 > 2 > 3.

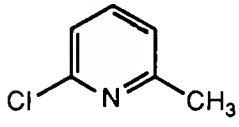
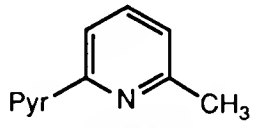
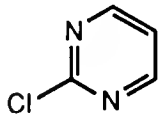
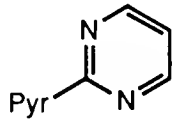
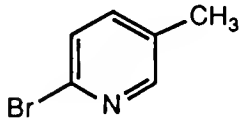
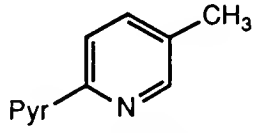
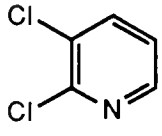
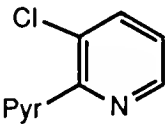
Substituted 2,3'-bipyridyls and a pyridylpyrimidine were prepared by palladium catalyzed coupling of commercially available diethyl(3-pyridyl)borane with heteroaryl halides. In the reported procedures, coupling reactions were carried out in THF with hydroxide as a base and tetramethylammonium ion as a phase transfer catalyst [15 - 18]. This system gave us variable yields. The solids tended to form a sludge in the bottom of the reaction vessel and the hydroxide was probably not effectively getting into solution. A two phase system consisting of a 2 to 1 mixture of THF and water containing 2-3 equivalents of potassium carbonate was used instead. This is similar to the system method employed the coupling of thiopheneboronic acids with halothiophenes [14]. Only 0.1 of an equivalent of palladium catalyst is required for effective coupling. This procedure was used to prepare 6-methyl-2,3'-bipyridine (6-12), 2-(3-pyridyl)pyrimidine (6-13),

5-methyl-2,3-bipyridine (6-14), and 3-chloro-2,3'-bipyridine (6-15) (eq 6-3 and Table 6-1). The reaction is very clean and usually gives high yields of coupled products.



(6-3)

Table 6-1. Compounds Prepared by Pd(0) Catalyzed Cross-coupling of Diethyl(3-pyridyl)borane with Heteroaryl Halides According to eq 6-3.

Het - X	Product	Yield
	 6-12	94% (crude) 85% (after chromatography)
	 6-13	72% (crude) 23% (recrystallized)
	 6-14	85% (crude)
	 6-15	30% (recrystallized)

Pyr = 3-pyridyl ring

CHAPTER 7
NATURE OF BINDING ENVIRONMENT OF SUBSTRATES IN SDS MICELLES AS REVEALED BY PROTON CHEMICAL SHIFTS AND LONGITUDINAL RELAXATION TIMES (T_1)

Introduction

Our interest in the binding sites of receptors sparked an interest in physical methods that could be used to explore the nature of binding environments. Because biological systems are so complex, we explored some possibilities in micellar model systems. Micelles serve as primitive models for membranes and lipid bilayers. Because of their ability to solubilize hydrophobic molecules in aqueous solutions, micelles are useful in reaction media and in industrial applications.

Surfactants are made up of amphiphilic molecules which have a polar head group that may be ionic and a non-polar, generally hydrocarbon, tail. In aqueous solution, surfactants aggregate so that the hydrophobic tails are associated and the polar head groups are interacting with the water. These micelles are dynamic entities. Monomers constantly diffuse in and out and micelles dissociate and reform. The outside of a micelle with ionic headgroups is surrounded by a layer of water and counter ions which is called the Stern layer [20, 21]. Figure 7-1 shows a cross

section of a model for a spherical micelle with anionic head groups. A micelle may not be as organized as the figure implies. There is still much debate over the exact structure of micelles [22 - 25] and the degree to which water can penetrate into its hydrophobic core [26]. Aqueous micellar solutions have the ability to solubilize hydrophobic compounds [20].

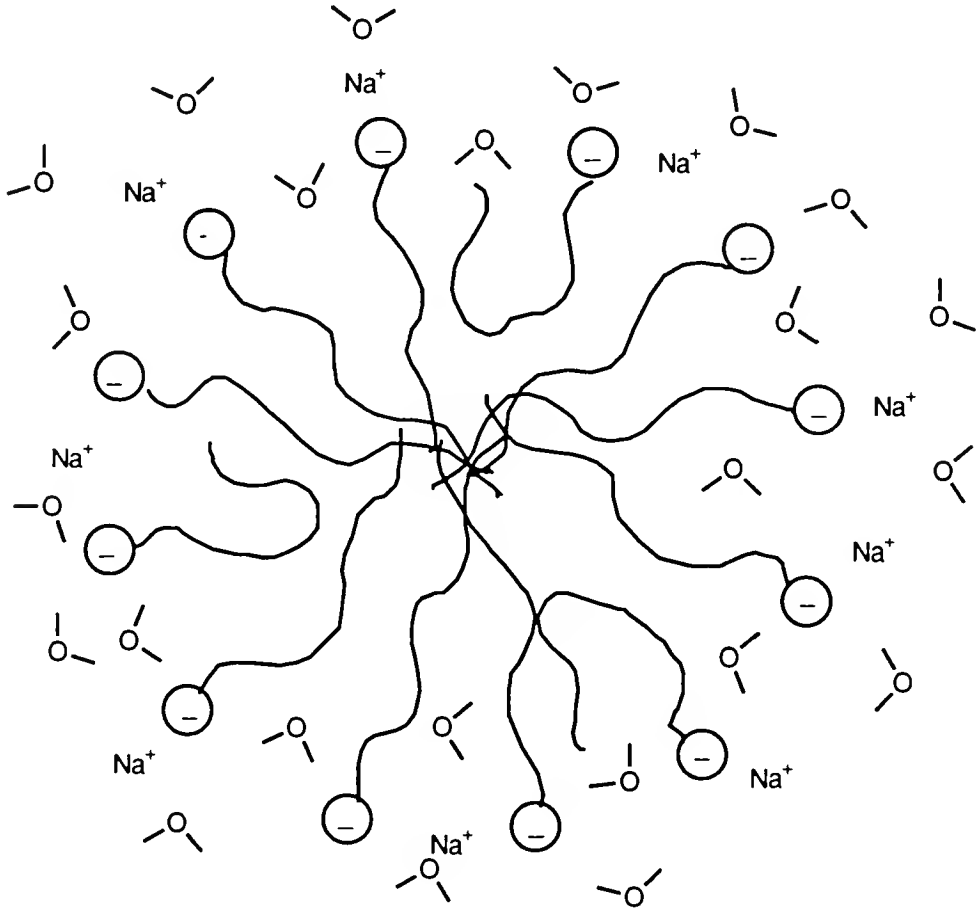


Figure 7-1. Hypothetical model for a spherical micelle.

Proton NMR techniques have been used to probe micellar systems for information about the environment of a substrate

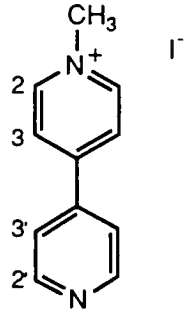
on binding [103 - 106]. Both the proton chemical shifts and the proton longitudinal relaxation times (T_1's) of a substrate are very sensitive indicators that relay information about its environment. Chemical shifts are sensitive to the polarity of the medium. Proton T_1's are sensitive to many factors including temperature, viscosity, molecular motions, and the presence of paramagnetic species [107, 108].

When a substrate binds to a micelle or macromolecule the correlation time (τ_c) for molecular motions is decreased and this leads to decreases in longitudinal relaxation times of the nuclei [107]. The binding of a substrate to a micelle is a dynamic process. The observed relaxation rates for such a system are a population weighted average of the relaxation rates for bound substrate and for free substrate [107].

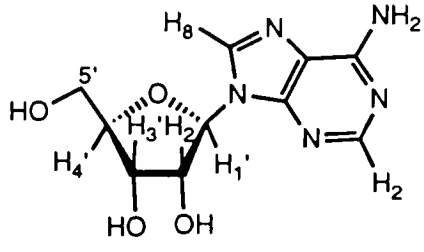
The surfactant used in this study was sodium dodecylsulfate (SDS) because the properties of aqueous micellar solutions of SDS have been extensively studied. An anionic sulfate head group and a 12 carbon aliphatic chain are present in SDS. Its aggregation numbers [109 - 113], CMC values [114], rates of diffusion of monomers and dissociation of micelles have been measured [115].

Binding of three substrates, 1-methyl-4,4'-bipyridinium ion (7-1), adenosine (Ado), and 2',3'-isopropylideneadenosine (iAdo), was investigated. 1-Methyl-4,4'-bipyridinium ion has a positively charged pyridium ring which would be expected to interact with the negatively charged head groups of SDS and a

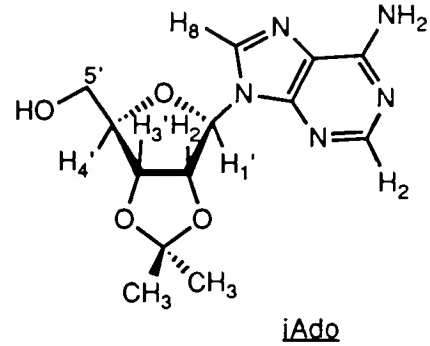
second more hydrophobic pyridine ring which may interact favorably with the hydrophobic interior of the micelle. Adenosine and its isopropylidene derivative are neutral molecules of very similar structure, consisting of a purine base attached by nitrogen 9 to the 1' carbon of the ribose ring. A distribution coefficient for Ado between aqueous and SDS micellar phases has been measured by high performance capillary electrophoresis [116]. Because the 2' and 3' hydroxyl groups of the isopropylidene derivative are functionalized as a cyclic acetal it is more hydrophobic than adenosine and may therefore have a higher affinity for the micellar phase.



7-1



Ado



iAdo

Nickel ion was used as a probe to gain additional information about the binding environment of the substrates with SDS and structure on binding. Nickel (II) contains an unpaired electron. When placed in a magenetic field, electrons produce a much stronger magnetic field than nuclei such as protons. The field due to the unpaired electron

greatly increases relaxation rates for other nuclei which come in contact with it [107, 117].

Because nickel is a dication it interacts strongly with the negatively charged surface of the SDS micelle. Binding constants of Ni^{2+} with SDS have been reported [118].

Nitrogen atoms can coordinate with the nickel ion; this leads to enhanced proton relaxation rates. Binding constants (K_b) for the purine nitrogens of adenosine with Ni^{2+} have been estimated to be as follows log K_B = 0.9 for N7 and 0.6 for N1 [119]. In addition, the hydroxyl groups of the ribose ring may also interact weakly with the nickel ion. A substrate may bind to SDS micelles in such a way that interactions which occur between the substrate and Ni^{2+} in purely aqueous solution are blocked or altered in aqueous micellar solutions. These changes may give further insight into the binding environment of a substrate with SDS.

In our study, comparisons were made between the chemical shifts and relaxation times (T_1's) of a given substrate in purely aqueous solutions and in aqueous solutions containing SDS micelles. In addition, paramagnetic nickel ion was added to solutions of the substrates in the presence and absence of SDS to determine if the interactions between substrate and nickel were changed on going to micellar solutions. Substrate concentrations were kept the same in both purely aqueous and micellar solutions so that concentration effects would not be present and direct comparisons could be made.

Results

Chemical Shift Differences

1-Methyl-4,4'-bipyridinium iodide

Large chemical shift changes were observed for 7-1 in aqueous solution when SDS micelles were present. Protons were deshielded compared with purely aqueous solutions (Table 7-1). By contrast addition of Ni^{2+} to solutions of 7-1 with and without SDS had no effect on the chemical shifts of the substrate or SDS.

Table 7-1. Chemical Shifts of 1-Methyl-4,4'-bipyridinium Iodide in D_2O with and without SDS and Ni^{2+} at 25.0 °C.

	Chemical Shifts / ppm[a]			
	No SDS		0.18 M SDS	
Proton	No Ni^{2+}	1.9 mM Ni^{2+}	No Ni^{2+}	1.5 mM Ni^{2+}
H2'	8.792	8.791	8.842	8.842
H3'	7.932	7.931	8.009	8.009
H3	8.408	8.406	8.520	8.520
H2	8.925	8.923	9.056	9.056
NCH_3	4.464	4.463	4.514	4.513

[a] TSP was used as an internal standard.

Chemical shifts were measured for 1-methyl-4,4'-bipyridinium iodide under several conditions; in D_2O at 2 different concentrations, in CD_3OD, and in D_2O containing 0.18 M SDS. In aqueous solutions, chemical shifts for 7-1 move

slightly upfield as the concentration of substrate is decreased (Table 7-2). This shielding may be due to increased dissociation of cation and counter anion of an ion pair resulting in a change in solvation on dilution [120]. A decrease in stacking interactions of the aromatic rings on dilution would give shifts that were deshielded as observed in the case of nucleotide bases [121].

Table 7-2. Proton Chemical Shift Differences For Solutions of 1-Methyl-4,4'-bipyridinium Iodide in Various Media Compared with a 30 mM Aqueous Solution.

	Chemical Shift Differences / ppm[a]		
Proton	δ_{D2O}[b] - δ_{D2O}	δ_{SDS}[c] - δ_{D2O}	δ_{CD3OD}[d] - δ_{D2O}
H2'	-0.014	0.050	0.047
H3'	-0.007	0.077	0.079
H3	-0.010	0.112	0.118
H2	-0.013	0.131	0.138
NCH_3	-0.015	0.050	0.028

[a] Negative shift differences indicate shielding relative to a 30 mM aqueous solution of 7-1.
[b] 5.1 mM 7-1 in D_2O.
[c] 30 mM 7-1 and 0.18 M SDS in D_2O.
[d] 42 mM 7-1 in CD_3OD.

A 42 mM methanolic solution of 7-1 gave signals that were shifted downfield relative to those of an aqueous solution containing 30 mM substrate (Table 7-2). The aromatic protons nearest to the positively charged pyridinium nitrogen show the greatest change. The magnitude of this

change decreases as the distance from the charged nitrogen increases. For example, the H2 protons show a shift difference of 0.138 ppm while the H2' protons show a difference of only 0.047 ppm. Less polar methanol does not solvate the positively charged nitrogen as well as water and so this atom has a larger deshielding effect in methanol [122]. The methyl protons are not deshielded as much as the aromatic protons α to the quaternized nitrogen. The shift difference for the methyl protons is 0.028 ppm and that for the H2 protons is 0.138 ppm. Medium effects may be different on protons which are attached to sp^2 hybridized carbons and sp^3 hybridized carbons; perhaps this accounts for the unequal changes at the two sites.

Chemical shifts of 7-1 in a 0.18 M aqueous solution of SDS are also deshielded relative to a purely aqueous solution (Table 7-2) at the same substrate concentration (30 mM). Again, the aromatic protons nearest to the positively charged nitrogen show the greatest shift. The shift difference for H2 is 0.131 ppm while that for H2' is 0.050 ppm. This result suggests that 7-1 is in an environment which is less polar than water when present in micellar SDS solution.

Comparison of the spectra of 7-1 in methanol-d_4 at a concentration of 42 mM and in 0.18 M aqueous solution of SDS at a concentration of 30 mM shows very similar chemical shifts for the aromatic protons. However, the observed chemical shifts for protons of 7-1 in SDS solution are a weighted average between the chemical shifts of substrate in

aqueous and micellar phases. Because all of the substrate may not be bound to SDS, the chemical shifts of the completely bound substrate may be even further downfield than observed. Therefore, the similarity in shifts between the substrate in methanolic solution and in SDS solution is coincidental and does not necessarily suggest that the polarity of SDS is similar to that of methanol. In contrast to the similarity in shifts between the aromatic protons in methanol and in SDS solution, the shifts of the N-methyl group show a difference. The N-methyl is more deshielded in SDS solution relative to methanol. This may indicate that the N-methyl group is in a slightly different environment than the aromatic protons. Perhaps it is near the polar head groups of SDS.

Purine nucleosides

Chemical shift changes for protons of Ado and iAdo are more modest than those of 7-1 on going from purely aqueous to aqueous solutions containing SDS (Tables 7-3 and 7-4). Neutral molecules would not be expected to show as big a change as charged molecules when the polarity of the medium is decreased. Both upfield and downfield shifts are observed for Ado and iAdo (Table 7-5). Changes in shifts of iAdo are greater than changes for Ado as would be expected if iAdo had a higher affinity for the SDS micelles than Ado. For example, the shift difference on going from water to SDS is 0.012 and 0.026 ppm for H8 and H2 of Ado, respectively and

Table 7-3. Chemical Shifts of 20 mM Adenosine Solutions in D_2O with and without SDS and Ni^{2+} at 25.0 °C.

	Chemical Shifts / ppm[a]			
	No SDS		0.12 M SDS	
Proton	No Ni^{2+}	1.0 mM Ni^{2+}	No Ni^{2+}	1.0 mM Ni^{2+}
H8	8.308	8.308	8.320	8.320
H2	8.185	8.185	8.211	8.211
H1'	6.052	6.050	6.042	6.042
H3'	4.438	4.435	4.444	4.443
H4'	4.304	4.303	4.292	4.291
H5'	3.891	3.889	3.884	3.882

[a] TSP was used as an internal standard.

Table 7-4. Chemical Shifts of 20 mM 2',3'-Isopropylideneadenosine Solutions in D_2O with and without SDS and Ni^{2+} at 25.0 °C.

	Chemical Shifts / ppm[a]			
	No SDS		0.12 M SDS	
Proton	No Ni^{2+}	1.0 mM Ni^{2+}	No Ni^{2+}	1.0 mM Ni^{2+}
H8	8.272	8.268	8.321	8.322
H2	8.197	8.192	8.237	8.238
H1'	6.213	6.207	6.181	6.180
H2'	5.402	5.400	5.339	5.341
H3'	5.121	5.118	5.096	5.095
H4'	4.509	4.507	4.485	4.483
H5'	3.805	3.804	3.828	3.829
CH_3 exo	1.684	1.683	--	--
CH_3 endo	1.456	1.456	1.426	1.425

[a] TSP was used as an internal standard.

0.049 and 0.040 ppm for H8 and H2 of iAdo, respectively. Again, no changes in chemical shifts are observed when Ni^{2+} is added.

Table 7-5. Proton Chemical Shift Differences For Solutions of 20 mM Ado and 20 mM iAdo in 0.18 M Aqueous SDS Compared with 20 mM Ado and 20 mM iAdo Aqueous Solutions.

	Shift Difference / ppm[a]	
	Ado	iAdo
Proton	$\delta_{SDS} - \delta_{D2O}$	$\delta_{SDS} - \delta_{D2O}$
H8	0.012	0.049
H2	0.026	0.040
H1'	-0.010	-0.029
H2'	--	-0.063
H3'	0.006	-0.025
H4'	-0.012	-0.024
H5'	-0.030	0.023
CH_3 endo	--	-0.030

[a] Negative shift differences indicate shielding relative to 20 mM aqueous solutions of Ado and iAdo.

Proton Longitudinal Relaxation Rates

The proton longitudinal relaxation times or T_1's were measured for substrates in D_2O solution with and without SDS micelles and paramagnetic nickel ion. Nonselective T_1's were measured using the inversion-recovery pulse sequence [108, 123, 124] with the temperature regulated at 25.0 °C.

All of the T_1 values were measured in a single experiment usually using 10 different delay times between the inversion and observation pulses. Rather than making comparisons between T_1 values which are inversely related to the relaxation rates, comparisons are made between relaxation rate constants, R_1 where $R_1 = 1/T_1$, which vary in the same manner as relaxation rates and are more easily understood.

1-Methyl-4,4'-bipyridinium iodide

A solution containing 30 mM 7-1 and 0.18 M SDS in D_2O gave faster proton relaxation rates for 7-1 than a solution containing only 7-1 in D_2O (Table 7-6). The relaxation rate constants for H2 and H2' in D_2O are 0.270 and 0.222 s^{-1}, respectively and in 0.18 M SDS solution these values increase to 0.838 and 0.689 s^{-1}, respectively. The increase in relaxation rates for 7-1 in SDS solution indicates that 7-1 binds to the micelles.

A solution that contained 1.9 mM Ni^{2+} and 29 mM 7-1 gave faster relaxation rates for all of the protons than a solution which contained only 30 mM 7-1 (Table 7-6). The relaxation rate constants for H2' went from 0.222 to 2.14 s^{-1} and those for H2 increased from 0.270 to 0.446 s^{-1} on addition of Ni^{2+}. The protons closest to the free nitrogen atom show the greatest increase two reasons: (1) The lone pair electrons of the unquaternized nitrogen atom are free to coordinate with the metal ion thereby concentrating the nickel ion at this site. (2) Electrostatic interactions

between the positively charged pyridine and nickel ion are unfavorable and therefore minimize interaction.

Table 7-6. Proton Longitudinal Relaxation Rate Constants for 1-Methyl-4,4'-bipyridinium Iodide in D_2O with and without Nickel and SDS at 25.0 °C.

	Relaxation Rate Constants ($R_1=1/T_1$) / s^{-1}			
	No Ni^{2+}		with Ni^{2+}	
Proton	D_2O[a]	0.18 M SDS[a]	D_2O[b]	0.18 M SDS[c]
H2'	0.222	0.689	2.14	10.2
H3'	0.296	1.01	1.44	6.85
H3	0.303	0.984	0.880	3.88
H2	0.270	0.838	0.446	2.18
NCH_3	0.689	1.43	0.742	2.51

[a] 30 mM 7-1.
[b] 29 mM 7-1 and 1.9 mM Ni^{2+}.
[c] 29 mM 7-1 and 1.5 mM Ni^{2+}.

The presence of 1.5 mM Ni^{2+} in 0.18 M SDS and 29 mM 7-1 enhanced the proton relaxation rates of both the substrate and SDS (Table 7-6). The relaxation rate constant for H2' of the substrate increased from 0.689 s^{-1} to 10.2 s^{-1} and that for H2 increased from 0.838 s^{-1} to 2.18 s^{-1}. For SDS, the relaxation rate constants for protons at positions 1, 2, and 12 increased from 1.22, 1.56, 0.800 s^{-1}, respectively, to 4.00, 3.55, and 1.47 s^{-1}, respectively. Even the proton in the 12 position in SDS is relaxed by nickel indicating that either water is able to substantially penetrate the micelle or the SDS monomers in the micelle are packed in such a way

to expose some of the methyl groups to the surface near the water interface.

The increase in the observed relaxation rate constants for SDS shows that Ni^{2+} is bound to the charged surface of the micelle as expected. The nickel dication has a binding constant of about 500 M^{-1} with SDS [118]. Thus, nearly all of the Ni^{2+} (99%) will be associated with the negatively charged surface of the SDS micelles rather than be free in solution. Because most of the Ni^{2+} is associated with the micelle, enhancement of the relaxation rates for substrate indicates that the substrate must also be interacting with the SDS micelles.

The larger relaxation rate constants for 7-1 in SDS solution with nickel over those in bulk water is a result of the reduced volume of the micellar phase. In the SDS solution, both nickel and substrate are in the micellar phase which is much smaller in volume than that of the bulk solution so that the relative concentrations of the two species in the micellar phase are higher. If both the nickel and substrate were in the aqueous phase rather than the micellar phase, the solution concentrations would be similar to the concentrations in the sample without SDS and the corresponding relaxation rates would be expected to be similar. If the nickel were associated with the SDS and 7-1 were free in solution then the effective concentration of the nickel in solution would be low and the proton relaxation

rates would be decreased relative to the sample containing no SDS. The same result would be observed if the opposite were true and the substrate was bound but the nickel was free in solution.

Relative relaxation rates (Table 7-7) for aromatic protons of 7-1 in D_2O normalized to the value for the H2 proton are instructive. They are very similar to those normalized values for D_2O containing 0.18 M SDS. In contrast, the relative relaxation rate for the N-methyl group is decreased from 2.55 in D_2O to 1.71 in aqueous SDS solution. This decrease may be due to a predominant structure for substrate bound to SDS which selectively increases the relaxation rates of the aromatic protons.

Table 7-7. Relative Proton Longitudinal Relaxation Rates for 1-Methyl-4,4'-bipyridinium Iodide in D_2O with and without Nickel and SDS at 25.0 °C.

	Relative Relaxation Rates			
	No Ni^{2+}		with Ni^{2+}	
Proton	D_2O[a]	SDS[b]	D_2O[c]	SDS[d]
H2'	0.822	0.822	4.80	4.68
H3'	1.10	1.21	3.23	3.14
H3	1.12	1.17	1.97	1.78
H2	1.00	1.00	1.00	1.00
NCH_3	2.55	1.71	1.66	1.15

[a] 30 mM 7-1.
[b] 30 mM 7-1 and 0.18 M SDS.
[c] 29 mM 7-1 and 1.9 mM Ni^{2+}.
[d] 29 mM 7-1, 0.18 M SDS, and 1.5 mM Ni^{2+}.

Solutions of 7-1 in D_2O with Ni^{2+} and in D_2O with SDS and Ni^{2+} (Table 7-7) also show similar relative relaxation rates for the aromatic protons. Both the magnitudes of the relative relaxation rates as well as the order have changed in comparison with solutions which do not contain Ni^{2+}. The protons nearest to the free nitrogen now show the greatest relative rates and the rate falls off with distance from this site. The interaction of the paramagnetic nickel ion with the free nitrogen is responsible for these new patterns. Again, the relative relaxation rate of the N-methyl is decreased in the solution containing SDS. This decrease is due to interactions with SDS and not to an effect of the Ni^{2+} because the magnitude of the change is similar to that observed in solutions in the absence of Ni^{2+}.

Purine nucleosides

The proton relaxation rate constants for Ado are only slightly increased on going from aqueous to solutions micellar SDS indicating that only a small fraction is bound (Table 7-8). The relaxation rate constants for the purine protons H8 and H2 showed almost no change and only went from 0.594 to 0.596 s^{-1} and from 0.200 to 0.226 s^{-1}, respectively, on going from D_2O to 0.12 M SDS. A bigger increase was seen for the sugar protons. The H1' proton went from 0.420 to 0.514 s^{-1}, the H4' proton from 0.592 to 0.816 s^{-1}.

Table 7-8. Proton Longitudinal Relaxation Rate Constants for 20 mM Adenosine Solutions in D_2O with and without Nickel and SDS at 25.0 °C.

	Relaxation Rate Constants ($R_1=1/T_1$) / s^{-1}			
	No Ni^{2+}		with 1.5 mM Ni^{2+}	
Proton	D_2O	0.12 M SDS	D_2O	0.12 M SDS
H8	0.594	0.596	1.40	2.71
H2	0.200	0.226	1.24	2.65
H1'	0.420	0.514	0.778	1.45
H3'	0.682	0.726	0.935	1.63
H4'	0.592	0.816	0.828	1.35
H5'	2.03	2.40	2.31	3.00

The proton relaxation rates for iAdo on going from D_2O to SDS solution show a greater increase than those for Ado (Table 7-9). The relaxation rate constant for H8 increased from 0.371 to 0.834 s^{-1} and that for H2 increased from 0.090 to 0.457 s^{-1}. As expected, the more hydrophobic iAdo seems to have a greater affinity for the SDS micelles than Ado. Relaxation rates could not be measured for the endo and exo methyls of the isopropylidene group because they were overlapped by signals from SDS.

When Ni^{2+} was added to solutions of Ado in D_2O and in D_2O with SDS, the proton relaxation rates increased (Table 7-8). The same result was observed for solutions of iAdo in the presence of Ni^{2+} (Table 7-9). Again, the relaxation rates are greater in the solution containing SDS because of the reduced micellar volume and the higher concentration of nickel.

Whereas the changes in relaxation rates for Ado on going from aqueous to micellar solutions do not strongly support the conclusion that association occurs, the changes seen in the solution containing Ni^{2+} are more convincing. Thus, nickel ion is a sensitive probe for determining whether a substrate interacts with the micelle.

Table 7-9. Proton Longitudinal Relaxation Rate Constants for 20 mM 2',3'-Isopropylideneadenosine Solutions in D_2O with and without Nickel and SDS at 25.0 °C.

	Relaxation Rate Constants ($R_1=1/T_1$) / s^{-1}			
	No Ni^{2+}		with 1.5 mM Ni^{2+}	
Proton	D_2O	0.12 M SDS	D_2O	0.12 M SDS
H8	0.371	0.834	1.12	6.06
H2	0.090	0.457	1.01	6.41
H1'	0.409	0.936	0.700	2.62
H2'	0.702	1.18	0.939	2.81
H3'	0.705	1.34	0.950	2.57
H4'	0.500	1.12	0.721	2.37
H5'	1.74	2.93	2.27	5.00
CH_3 exo	1.40	--	1.46	--
CH_3 endo	1.60	--	1.67	--

Examination of relative proton relaxation rates for Ado in solutions containing Ni^{2+} (Table 7-10) shows there is only a difference in the relative rate of relaxation for the H5' protons on going from water to SDS. This change is not observed in the absence of nickel and indicates that

interactions of Ado with Ni^{2+} are changed on binding to SDS so that this site is no longer as accessible to nickel.

Table 7-10. Relative Proton Relaxation Rate Constants for 20 mM Adenosine Solutions in D_2O with and without Nickel and SDS at 25.0 °C.

	Relative Relaxation Rates			
	No Ni^{2+}		with 1.5 mM Ni^{2+}	
Proton	D_2O	0.12 M SDS	D_2O	0.12 M SDS
H8	1.41	1.16	1.80	1.87
H2	0.476	0.440	1.59	1.83
H1'	1.00	1.00	1.00	1.00
H3'	1.62	1.41	1.20	1.12
H4'	1.41	1.59	1.06	0.93
H5'	4.83	4.67	2.97	2.07

Differences in relative relaxation rates for iAdo (Table 7-11) are observed both on binding to SDS and in interactions with nickel in the presence and absence of SDS. On binding to SDS the H5' protons do not relax to the same extent as the other protons both in the presence and absence of Ni^{2+}. The relative relaxation rate of the H2' proton is enhanced on binding to SDS. When Ni^{2+} is added to a solution containing SDS, the relaxation of the H8 proton is selectively enhanced.

Discussion

It is tempting to draw too many conclusions from the results given above and to define exactly the environments of

substrates on binding. These results are only preliminary. The following conclusions presented are those which are indicated by the data collected so far but will most likely be modified and refined after more work.

Table 7-11. Relative Proton Relaxation Rate Constants for 20 mM 2',3'-Isopropylideneadenosine Solutions in D_2O with and without Nickel and SDS at 25.0 °C.

	Relative Relaxation Rates			
	No Ni^{2+}		with 1.5 mM Ni^{2+}	
Proton	D_2O	0.12 M SDS	D_2O	0.12 M SDS
H8	0.742	0.745	1.55	2.56
H2	0.180	0.408	1.40	2.70
H1'	0.814	0.836	0.971	1.11
H2'	1.40	1.05	1.30	1.19
H3'	1.41	1.20	1.32	1.08
H4'	1.00	1.00	1.00	1.00
H5'	3.48	2.62	3.15	2.11
CH_3 exo	2.80	--	2.02	--
CH_3 endo	3.20	--	2.31	--

Binding Environment of 1-Methyl-4,4'-bipyridinium Iodide

The chemical shift changes for the protons of 7-1 on going from purely aqueous solution to aqueous micellar solution indicate that the substrate is interacting with the micelles. Moreover, this interaction seems to place the

substrate in an environment which is less polar than pure water. The aromatic protons show chemical shifts in 0.18 M aqueous SDS solution which are similar to those observed for the substrate in methanol-d_4 while the shifts of the N-methyl group differs. This may indicate that the two regions of the molecule are experiencing different environments. An example of a binding structure which would place the two ends of the molecule in different enviroments is given in Figure 7-2.

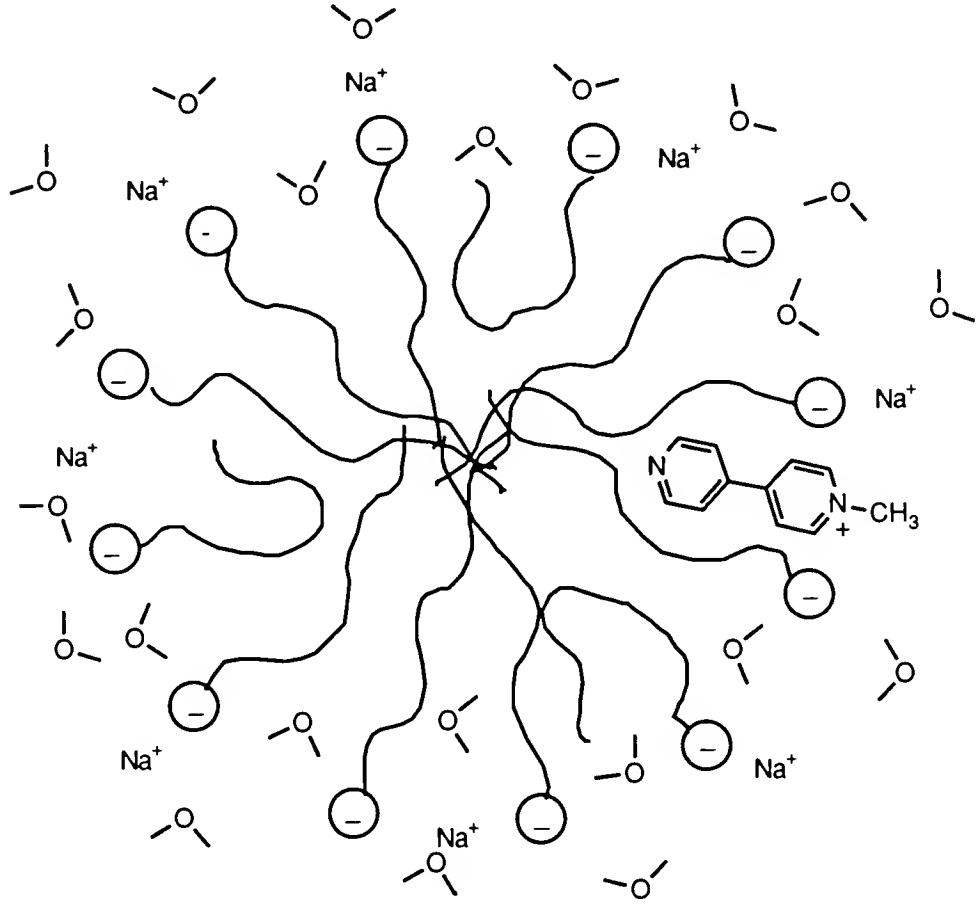


Figure 7-2. Possible model for binding of 1-methyl-4,4'-bipyridinium ion with SDS micelles.

Changes in the relaxation rates of the protons of 7-1 support the conclusions drawn from the variations in chemical shifts. The increase in proton relaxation rate constants for 7-1 on going from purely aqueous to micellar SDS solutions also shows that the substrate is interacting with the micelle. The rate of relaxation of the methyl protons is not enhanced as much as those for the aromatic protons. This may indicate that the predominant structure on binding is one where the aromatic protons would be selectively enhanced such as Figure 7-2. However, care must be taken in interpretation of these differences because protons bound to an sp^2 hybridized carbon may be affected differently than protons bound to an sp^3 hybridized carbon where there are multiple protons, the major contributers to relaxation.

The similarity of the relative relaxation rates of the protons of 7-1 in aqueous solution containing nickel and in aqueous micellar solutions containing nickel seems to suggest that the substrate is in a similar largely aqueous environment in both experiments. This contradicts conclusions drawn from chemical shift differences which suggest that the substrate is in a less polar medium than water when in SDS solutions and differences in relaxation rates which show that the substrate is bound to SDS. However, the observed relaxation rates are a weighted average of the rates for substrate free in solution, substrate bound to SDS, and substrate interacting with paramagnetic nickel. It was hoped that binding of the substrate to SDS would

change the interactions between substrate and nickel and that these changes would be reflected in changes in T_1 values. Unfortunately, relaxation induced by nickel binding to substrate is so great that these subtle changes in accessibility are overwhelmed.

Proton chemical shifts and proton longitudinal relaxation rates are very sensitive reporters of a molecule's environment. Changes in both for 7-1 on going from aqueous to micellar solutions clearly show that substrate is binding to SDS. Differential effects on the chemical shifts and on the relaxation rates of the aromatic and methyl protons of 7-1 suggest that they are located in different environments. These measurements have allowed us to make a prediction about the binding structure. Thus, measurements of proton chemical shifts and longitudinal relaxation rates appear useful in determining the nature of binding environments.

Binding Environments of the Purine Nucleosides

Less can be inferred about the binding environments of the purine nucleosides. The changes both in chemical shifts and in relaxation rates for Ado and iAdo are small in comparison to those observed for 7-1 and they more complex. The differential effects on the protons within a given substrate are hard to interpret without a better understanding of the total changes expected when a substrate binds completely to SDS micelles. However, their changes in

chemical shift and increase in relaxation rates when added to SDS solution show that they are indeed binding. The changes are larger for iAdo than Ado suggesting that iAdo has a greater affinity for the micelles than Ado. The large change in rate produced by nickel demonstrates conclusively that both substrates bind to SDS. Adding Ni^{2+} would therefore seem to be a method of choice to show that weakly binding substrates do in fact bind to SDS micelles.

CHAPTER 8
EXPERIMENTAL

Instrumentation

Proton and carbon NMR spectra were recorded on either a Varian VXR-300 or General Electric QE-300. Ultraviolet and visible spectra were recorded on a Perkin-Elmer 330 spectrophotometer. Measurements of pH were made with a Radiometer PHM 64 pH meter using Bates' buffers for standardization [125]. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

Reagents

All chemicals were reagent grade and purchased from various suppliers. Solutions of 1.5 M LDA in cyclohexane were purchased from Aldrich Chemical Co. and used without standardization. Internal standards used in NMR solutions include tetramethylsilane (TMS), sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS), and sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP).

Preparations

1'-Methyl-2,3'-bipyridinium Iodide (5-2). To a solution of 2,3'-bipyridine in 2 mL of acetone was added excess methyl iodide. After sitting overnight at room temperature, the reaction mixture was diluted with 1 mL of acetone and 0.664 g of pale yellow solid was removed by filtration (mp 165-166.5 °C). Recrystallization from water and isopropyl alcohol yielded 0.555 g (1.86 mmol) of pale yellow crystals (mp 166.5-168.5 °C, lit. mp 167-168 °C [75]). ^{1}H NMR (D_2O, TSP) δ 9.374 (H2', 1 H, s), 9.011 (H4', 1 H, d, $J_{4,5}$ = 8.2 Hz), 8.878 (H6', 1 H, d, $J_{5,6}$ = 6.1 Hz), 8.761 (H6, 1 H, ddd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.7 Hz, $J_{3,6}$ = 0.95 Hz), 8.197 (H5', 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 6.1 Hz), 8.112 (H4, 1 H, unsymmetrical td, $J_{3,4} \approx J_{4,5}$ = 7.7 Hz, $J_{4,6}$ = 1.7 Hz), 8.050 (H3, 1 H, dt, $J_{3,4}$ = 8.0 Hz, $J_{3,5} \approx J_{3,6}$ = 1.1 Hz), 7.641 (H5, 1 H, ddd, $J_{4,5}$ = 7.5 Hz, $J_{5,6}$ = 5.0 Hz, $J_{3,5}$ = 1.3 Hz), 4.527 (NCH_3, 3 H, s).

1,1'-Dimethyl-2,3'-bipyridinium Diiodide (5-3). A solution of 0.158 g (1.01 mmol) of 2,3'-bipyridine and 1 mL (16 mmol) of methyl iodide in 2 mL of acetonitrile was heated in a sealed vial on a steam bath for 4 hrs. A red-orange oil came out of solution which crystallized on standing to give 0.355 g of yellow solid after filtration. Recrystallization from water and ethanol yielded 0.298 g (0.677 mmol, 67% yield) of light yellow crystals (mp 210-214 °C decomp).

An analytical sample was prepared by two recrystallization from water and ethanol (mp 210-214 °C, decomp, lit mp 196-197 °C [75]). ^{1}H NMR (D_2O, TSP, peaks are broad and no fine coupling is present) δ 9.298 (H2', 1 H, s), 9.167 (H6 or H6', 1 H, d, $J_{5,6}$ = 6.1 Hz), 9.099 (H6 or H6', 1 H, d, $J_{5,6}$ = 6.2 Hz), 8.890 (H4', 1 H, d, $J_{4,5}$ = 8.3 Hz), 8.754 (H4, 1 H, t, $J_{3,4} = J_{4,5}$ = 7.7 Hz), 8.387 (H5 or H5', 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 6.3 Hz), 8.274 (H5 or H5', 1 H, unresolved dd), 4.565 (1-NCH_3, 3 H, s), 4.289 (1'-NCH_3, 3 H, s). Anal. Calcd. for $C_{12}H_{14}N_2I_2$: C, 32.75; H, 3.21; N, 6.37. Found: C, 32.70; H, 3.11; N, 6.27.

1'-(2-(2-Pyridyl)ethyl)-2,3'-bipyridinium Chloride Hydrochloride (5-6). A solution of 4.18 g (18.2 mmol) of 2,3'-bipyridine dihydrochloride and 4.0 mL (37 mmol) of 2-vinylpyridine in 120 mL of methanol was heated at reflux for 2 days. The solvent was evaporated to leave a yellow liquid and solid residue. Two recrystallizations of the yellow residue from a solution of 20% ethanol and 80% isopropyl alcohol gave 4.34 g of white solid (mp 185-188 °C, mp dependent on rate of heating). A second crop of 0.256 g of pale yellow solid (mp 181-186.5 °C) was collected (72% total yield). An analytical sample was prepared by recrystallization from isopropyl alcohol (mp 188-189 °C, decomp). Anal. Calcd. for $C_{17}H_{16}N_3Cl.HCl.H_2O$: C, 57.96; H, 5.44; N, 11.93. Found: C, 58.66; H, 5.47; N, 12.08. A second analytical sample was prepared by two recrystallizations from a solution of isopropyl alcohol

containing a couple drops of water (mp 191-193 °C, decomp). Anal. Calcd. for $C_{17}H_{16}N_3Cl.HCl.1/4H_2O$: C, 60.28; H, 5.21; N, 12.40. Found: C, 60.42; H, 5.41; N, 12.38.

1'-(2-(2-pyridyl)ethyl)-2,3'-bipyridinium Chloride (5-7). A solution of 4.15 g (11.8 mmol) of 5-6 in 100 mL of methanol was stirred with a suspension of 5.21 g (calcd. 50 mmol of monomer, 4 eq) poly-4-vinylpyridine resin overnight. After filtration to remove the resin, the methanol was evaporated to a yellow oil. Addition of hexanes to a solution of the oil in isopropyl alcohol produced 2.45 g of white precipitate (mp 41-52 °C). The mother liquor was evaporated to an oil which solidified on standing and was recrystallized from acetonitrile and ethyl acetate to yield 1.07 g of white solid (mp 54-65 °C). Recrystallization of both crops from acetonitrile and ethyl acetate yielded 2.80 g of white solid (mp 64.5-66.5 °C, 75% yield). An analytical sample was prepared by further recrystallization from acetonitrile and ethyl acetate (mp 66-67 °C). ^{1}H NMR (DMSO, TMS, A J resolve function was used on the FID to resolve coupling costants.) δ 9.836 (H2', 1H, t, $J_{2,4} \approx J_{2,6} = 1.3$ Hz), 9.218 (H4', 1 H, ddd, $J_{4,5} = 8.2$ Hz, $J_{2,4}$, $J_{4,6} = 1.9$ Hz, 1.2 Hz), 9.151 (H6', 1 H, dt, $J_{5,6} = 6.1$ Hz, $J_{2,6} \approx J_{4,6} = 1.3$ Hz), 8.813 (H6, 1 H, ddd, $J_{5,6} = 4.8$ Hz, $J_{4,6} = 1.8$ Hz, $J_{3,6} = 0.9$ Hz), 8.497 (H11, 1 H, ddd, $J_{5,6} = 4.9$ Hz, $J_{4,6} = 1.8$ Hz, $J_{3,6} = 0.9$ Hz), 8.288 (H3, 1 H, dt, $J_{3,4} = 8.0$ Hz, $J_{3,5} \approx J_{3,6} = 1.0$ Hz), 8.241 (H5', 1 H, dd, $J_{4,5} = 8.2$ Hz, $J_{5,6} = 6.0$ Hz), 8.098 (H4, 1 H, td, $J_{3,4} \approx J_{4,5} = 7.8$ Hz, $J_{4,6} = 1.8$ Hz), 7.787

(H13, 1 H, td, $J_{3,4} \approx J_{4,5}$ = 7.7 Hz, $J_{4,6}$ = 1.8 Hz), 7.603 (H5, 1 H, ddd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 4.8 Hz, $J_{3,5}$ = 1.0 Hz), 7.398 (H14, 1 H, dt, $J_{3,4}$ = 7.8 Hz, $J_{3,5} \approx J_{3,6}$ = 1.1 Hz), 7.298 (H12, 1 H, ddd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 4.9 Hz, $J_{3,5}$ = 1.2 Hz), 5.177 (H7, 2 H, t, J = 7.2 Hz), 3.587 (H8, 2 H, t, J = 7.2 Hz). Anal Calcd. for $C_{17}H_{16}N_3Cl \cdot H_2O$: C, 64.66; H, 5.74; N, 13.31. Found: C, 64.27; H, 5.93; N, 13.25.

1-Methyl-1'-(2-(1-methyl-2-pyridinio)ethyl)-2,3'-bipyridinium Triiodide (5-8). A solution of 212 mg (0.602 mmol) of 5-7 in 2.5 mL of acetonitrile was dried over molecular sieves for 2 days. After removing the sieves, 1.5 mL (24 mmol) of methyl iodide was added and the resulting solution was heated between 97-100 °C in a sealed tube for 2 hrs. A very viscous red-orange oil came out of solution on cooling. After decanting the solvent, the oil was crystallized from hot methanol with stirring as the solution cooled to yield 153 mg of yellow solid. Recrystallization from water and isopropyl alcohol gave 113 mg of light yellow crystals (mp 175-178 °C, decomp, 24% yield). ^{1}H NMR (DMSO, TMS) δ 9.687 (H2', 1 H, s), 9.417 (H6', 1 H, d, $J_{5,6}$ =6.3 Hz), 9.328 (H6, 1 H, d, $J_{5,6}$ = 6.0 Hz), 9.104 (H11, 1 H, d, $J_{5,6}$ = 6.0 Hz), 9.045 (H4', 1 H, d, $J_{4,5}$ = 8.4 Hz), 8.868 (H4, 1 H, t, $J_{3,4} \approx J_{4,5}$ = 8.0 Hz), 8.579 (H13, 1 H, partially overlaps 8.523, t, $J_{3,4} \approx J_{4,5}$ =7.8 Hz), 8.523 (H5', 1 H, partially overlaps 8.579, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 6.3 Hz), 8.390 (H5, 1 H, partially overlaps 8.349, unresolved dd), 8.349 (H3, 1 H, partially overlaps 8.390, d, $J_{3,4}$ = 8.4 Hz), 8.094 (H12, 1

H, partially overlaps 8.046, unresolved dd), 8.046 (H14, 1 H, partially overlaps 8.094, d, $J_{3,4}$ =8.1 Hz), 5.160 (H7, 2 H, t, J = 7.6 Hz), 4.431 (10-NCH_3, 3 H, s), 4.282 (1-NCH_3, 3 H, s), 3.961 (H8, 2 H, t, J = 7.5 Hz). Anal. Calcd. for $C_{19}H_{22}N_3I_3 \cdot H_2O$: C, 33.02; H, 3.50; N, 6.08. Found: C, 33.19; H, 3.43; N, 6.17.

Microscale Preparation of 1-Methyl-2,3'-bipyridinium Iodide from 5-8 Using Poly-4-vinylpyridine as a Base. Water was added dropwise to a mixture of 20 mg (0.029 mmol) of 5-8 in 3 mL of methanol until the solid dissolved. To the solution was added 40 mg of poly-4-vinylpyridine and the resulting suspension was stirred overnight. The yellow P-4VP was filtered out of the solution and the solvent was evaporated to a yellow solid. The solid was partially redissolved in a fresh portion of ethanol. The insoluble portion was filtered off and was insoluble in water. The ethanol was evaporated and an orange solid crystallized on standing (mp 144-146 °C). The melting point and ^1H NMR of this orange solid is consistent with the authentic 1-methyl-2,3'-bipyrdinium iodide prepared from 5-10. ^1H NMR (D_2O, DSS) δ 8.930 (H6, 1 H, d, $J_{5,6}$ = 6.4 Hz), 8.816 (H6', 1 H, d, $J_{5,6}$ = 5.1 Hz), 8.749 (H2', 1 H, d, $J_{2,4}$ = 2.3 Hz), 8.620 (H4, 1 H, unsymmetrical t, $J_{3,4} \approx J_{4,5} \approx 8$ Hz), 8.04-8.16 (H4', H3, H5, 3 H, m), 7.734 (H5', 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 5.0 Hz), 4.211 (1-NCH_3, 3 H, s).

Microscale Preparation of 1-Methyl-2,3'-bipyridinium Iodide from 5-8 Using Sodium Bicarbonate as a Base. To

0.80 mL of a solution of 0.2 M sodium bicarbonate was added 55 mg of 5-8. After 2 hrs, the solution was evaporated to leave a wet yellow solid. The solid was partially dissolved in ethanol and the insoluble material was removed. The ethanol was evaporated to a yellow oil which crystallized on standing to form a light brown solid (mp 140-151 °C). The ^1H NMR was consistent with 5-1.

1'-(2-(4-Nitrophenyl)ethyl)-2,3'-bipyridinium Iodide (5-9). A solution of 1.52 g (6.61 mmol) of 4-(2-bromoethyl)-1-nitrobenzene and 3 g (20 mmol) of sodium iodide in 10 mL of acetone was stirred overnight at room temperature to convert the bromide to the iodide. The sodium bromide precipitate was filtered out of the solution which was then evaporated. Ethyl acetate (20 mL) was added to the residue and the insoluble sodium iodide was removed by filtration. To the ethyl acetate filtrate was added 0.993 g (6.35 mmol) of 2,3'-bipyridine and the resulting solution was heated at reflux for 2 days. The product prepitated from the reaction mixture and was filtered to yield 1.84 g of bright yellow solid (mp 123.5 - 130.5 °C). Unreacted starting materials were detected in the filtrate by TLC. The volume of the filtrate was reduced by evaporation and the filtrate was heated at reflux for an additional day. A second crop of 0.478 g (mp 115-119 °C) of solid was collected to give a total yield of 2.32 g. TLC showed that unreacted starting materials were still present in solution. Both crops of product were recrystallized from acetonitrile and ethyl

acetate to give 1.87 g (4.32 mmol, 68% yield) of bright yellow needle-like crystals (mp 177-179 °C, decomp). ^{1}H NMR (DMSO, DSS) δ 9.855 (H2', 1 H, s), 9.298 (H4', 1 H, d, $J_{4,5}$ = 8.5 Hz), 9.158 (H6', 1 H, d, $J_{5,6}$ = 6.1 Hz), 8.869 (H6, 1 H, ddd, $J_{5,6}$ = 4.8, $J_{4,6}$ = 1.7, $J_{3,6}$ = 0.91 Hz), 8.25-8.40 (H3, H5', H11, H13, 4 H, m), 8.170 (H4, 1 H, td, $J_{3,4} \approx J_{4,5}$ = 7.8, $J_{4,6}$ = 1.8 Hz), 7.65-7.71 (H5, H10, H14, 3 H, m), 5.101 (H7, 2 H, t, 7.7 Hz), 3.586 (H8, 2 H, t, 7.6 Hz). ^{13}C NMR (DMSO) δ 150.18, 149.72, 146.63, 144.44, 144.33, 143.20, 142.44, 138.16, 138.10, 130.49 (C11, C13), 128.05, 125.23, 123.68 (C10, C14), 121.89, 61.05 (C7), 36.11 (C8).

A sample of the iodide was converted to the perchlorate salt by heating 92 mg (0.21 mmol) of 5-9 in aqueous sodium perchlorate. The perchlorate salt crystallized on cooling and was recrystallized from water and ethanol to give 53 mg (0.13 mmol, 62% yield) of flaky white crystals (mp 153-154.5 °C). The ^{1}H NMR of the perchlorate was consistant with that of the iodide. Anal. Calcd. for $C_{18}H_{16}N_3O_2 \cdot ClO_4$: C, 53.28; H, 3.97; N, 10.35. Found: C, 53.07; H, 3.87; N, 10.22.

1-Methyl-1'-(2-(4-nitrophenyl)ethyl)-2,3'-bipyridinium Diiodide (5-10). A total of 1.73 g (3.99 mmol) of 5-9 was divided into 4 parts and placed into four screw top test tubes (about 430 mg each). To each tube was added 4 mL of acetonitrile which was dried previously over 3A molecular sieves and 5 mL of methyl iodide which filled the tubes about halfway. The tubes were sealed with teflon caps and heated on a steam bath for 6.5 hours. A very viscous dark red oil

came out of solution and crystallized on cooling to give 2.06 g of dark red solid after filtration. Recrystallization from water and isopropyl alcohol gave 1.87 g (3.15 mmol, 79% yield) of dark red crystals (mp 176-180 °C. decomp). After drying under vacuum, the solid was lighter in color (mp 177-180 °C, decomp). ^{1}H NMR (DMSO, TSP) δ 9.556 (H2', 1 H, s), 9.26-9.34 (H6, H6', 2 H, overlapping d), 8.982 (H4', 1 H, d, $J_{4,5}$ = 8.3 Hz), 8.845 (H4, 1 H, td, $J_{3,4} \approx J_{4,5}$ = 7.9, $J_{4,6}$ = 1.2 Hz), 8.450 (H5', 1 H, dd, $J_{4,5}$ = 8.1, $J_{5,6}$ = 6.4), 8.372 (H5, 1 H, ddd, $J_{4,5}$ = 7.8, $J_{5,6}$ = 6.2, $J_{3,6}$ = 1.6 Hz), 8.300 (H3, 1 H, dd, $J_{3,4}$ = 7.9, $J_{3,5}$ = 1.2 Hz), 8.237 (H11, H13, 2 H, d, J = 8.7 Hz), 7.608 (H10, H14, 2 H, d J = 8.8 Hz), 5.026 (H7, 2 H, t, J = 7.8 Hz), 4.202 (NCH_3, 3 H, s), 3.543 (H8, 2 H, t, J = 7.8 Hz). Anal. Calcd. for $C_{19}H_{19}N_3O_2I_2 \cdot H_2O$: C, 38.47; H, 3.57; N, 7.08. Found: C, 38.19; H, 3.47; N, 6.92.

1-Methyl-2,3'-bipyridinium Perchlorate (5-1·ClO_4) from 5-10. A suspension of 1.77 g (2.99 mmol) of 5-10 in a solution of 0.849 g (6.01 mmol) of 2,2,6,6-tetramethylpiperidine and 25 mL of methanol was heated at reflux with stirring for 22 hrs. 5-10 dissolved slowly as it reacted. Dilution of the reaction mixture with 200 mL of ethyl acetate and cooling in a refrigerator overnight produced a dark brown precipitate. This precipitate was removed by filtration to give 0.609 g of 2,2,6,6-tetramethylpiperidine hydroiodide (mp>225 °C). The filtrate was concentrated on a rotary evaporator to give a black tar.

The tar was extracted with ethanol (10-15 mL) and then acetone. Both extracts were diluted with ethyl acetate and cyclohexane and chilled with stirring to produce a precipitate. Filtration of the precipitate from the ethanol gave 0.351 g of light brown product (mp 142-147 °C). A second crop of 0.189 g of dark brown solid (mp 140-145 °C) was precipitated by further dilution of the filtrate and cooling. The acetone extract yielded 31 mg of a dark brown precipitate (mp 143-147 °C). The brown precipitates consisted of a mixture of 5-1·I and 2,2,6,6-tetramethylpiperidine hydroiodide which could not be easily separated by recrystallization.

Purification was acheived by conversion of the iodide to the perchlorate hydroperchlorate salt. The combined precipitates (0.499 g) were dissolved in 10 mL of 4/1 methanol/ethanol and the volume of the solution was doubled by the addition of "magic" perchlorate (8/5/36 ethanol/70%$HClO_4$/ethyl acetate). A precipitate formed almost immediately after adding the perchlorate solution and filtration yielded 0.410 g of brown-orange solid product (mp>240 °C). ^1H NMR (D_2O, TSP) δ 9.00-9.04 (H2', H6', H6, 3 H, m), 8.688 (H4, 1 H, td, $J_{3,4} = J_{4,5} = 7.8$ Hz, $J_{4,6} = 1.0$ Hz), 8.629 (H4', 1 H, ddd, $J_{4,5} = 8.2$ Hz, $J_{2,4}$, $J_{4,6} = 2.2$, 1.5 Hz), 8.12-8.21 (H3, H5, H5', 3 H, m), 4.241 (NCH_3, 3 H, s).

The free base was prepared by stirring a solution of 0.410 g of the perchlorate hydroperchlorate in 40 mL of methanol and 2 mL of water with a suspension

poly-4-vinylpyridine for 2 hrs. The polymer was filtered from the solution and the orange filtrate was decolorized with charcoal. The methanolic solution was evaporated to yield 0.256 g of light yellow solid (mp 130-133 °C, decomp). Recrystallization from ethanol yielded 0.221 g (0.816 mmol, 27% yield from elimination from 5-10) of the perchlorate salt as light yellow crystals (mp 132-134 °C). An analytical sample was prepared by recrystallization once from a solution of ethanol and sodium perchlorate and once from ethanol (mp 132-134 °C, decomp). ^{1}H NMR (D2O, TSP) δ 8.964 (H6, 1 H, d, $J_{5,6}$ = 6.1 Hz), 8.843 (H6', 1 H, dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.5 Hz), 8.784 (H2', 1 H, dd, $J_{2,4}$ = 2.3 Hz, $J_{2,5}$ = 0.92 Hz), 8.655 (H4, 1 H, t, $J_{3,4} \approx J_{4,5}$ = 8.0 Hz), 8.04-8.19 (H4', H3, H5, 3 H, m), 7.767 (H5', 1 H, dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 5.1 Hz), 4.248 (NCH_3, 3 H, s). ^{13}C NMR (D_2O, TSP) δ 155.22, 154.14, 151.00, 149.58, 148.72, 140.87, 133.27, 131.26, 130.36, 127.33, 49.98. Anal. Calcd. for $C_{11}H_{11}N_2 \cdot ClO_4$: C, 48.81; H, 4.10; N, 10.35. Found: C, 49.00; H, 4.03; N, 10.29.

2,3'-Bipyridinium Hydroperchlorate (5-12). To a solution of 0.252 g (1.61 mmol) of 2,3'-bipyridine in about 3-5 mL of ethanol was added 8-9 drops of 70% $HClO_4$. A white precipitate formed on dilution with about 10 mL of ethanol and cooling. The precipitate was removed by filtration to give 0.298 g white solid. Recrystallization from ethanol gave 0.260 g of white crystals (mp 160-164 °C). ^{1}H NMR (D_2O, TSP) δ 9.234 (H2', 1 H, d, $J_{2,4}$ = 2.1 Hz), 8.933 (H4',

1 H, ddd, $J_{4,5}$ = 8.2 Hz, $J_{2,4}$ = 2.1 Hz, $J_{4,6}$ = 1.4 Hz), 8.849 (H6', 1 H, unresolved dd, $J_{5,6}$ = 5.7 Hz), 8.732 (H6, 1 H, ddd, $J_{5,6}$ = 5.1 Hz, $J_{4,6}$ = 1.7 Hz, $J_{3,6}$ = 0.95 Hz), 8.10-8.19 (H4, H5', 2 H, m), 8.042 (H3, 1 H, dt, $J_{3,4}$ = 8.0 Hz, $J_{3,5} \approx J_{3,6}$ = 1.1 Hz), 7.666 (H5, 1 H, ddd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 5.1 Hz, $J_{3,5}$ = 1.2 Hz). Anal. Calcd. for $C_{10}H_2N_2 \cdot 2HClO_4$: C, 46.80; H, 3.53; N, 10.92. Found: C, 46.45; H, 3.40; N, 10.68.

2,3'-Bipyridinium Dihydrochloride (5-13). Concentrated HCl was added to a solution of 5.0 mL of 2,3'-bipyridine in about 25 mL of isopropyl alcohol in an ice bath until cloudiness remained and a second phase formed. Ethanol was then added dropwise until the layers were miscible; a white solid precipitated. This hygroscopic solid (mp 230-234 °C, decomp., lit. mp 235-236 °C) was used without further purification in the preparation of 5-6. An analytical sample was prepared by recrystallization from a solution of ethanol with a couple drops of concentrated HCl. Anal. Calcd. for $C_{10}H_8N_2 \cdot 2HCl$: C, 52.42; H, 4.40; N, 12.23. Found: C, 52.45; H, 4.31; N, 12.16.

Yields of Lithium Enolate Salts and Their Hydrolysis-Decarboxylation Products. Following isolation from the reaction mixtures, the lithium salts were sticky and appeared to take up water on standing in air to give stable dry powders. No attempt was made to purify these salts which apparently formed in near quantitative yield. Yields are reported for the overall conversion of the aryl ester limiting reagent to the hydrolyzed-decarboxylated product.

Lithium Enolate of 3-nicotinoyl-2-piperidone (6-1).

Protected 1-trimethylsilyl-2-piperidone was prepared by the addition over 20 min of a solution of 5.68 g (57.3 mmol) of 2-piperidone in 15-20 mL of dry THF to a solution of 38 mL of 1.5 M LDA in cyclohexane (Aldrich) and 40 mL of dry THF at -70 °C under nitrogen. To the resulting amide anion was added 7.2 mL (56.7 mmol) of trimethylsilyl chloride and the solution was stirred at -70 °C for 15 min and then at room temperature for 2 hrs. Using the protected piperidone, 6-1 was prepared as described for 3-(5'-fluoronicotinoyl)-2-piperidone [97] using 38 mL (57 mmol) of 1.5 M LDA in cyclohexane and 5.2 mL (38 mmol) of ethyl nicotinate. Hydrolysis of the trimethylsilyl protecting group was accomplished by stirring the reaction mixture with 50 mL of water for 15 min. The lithium enolate 6-1 remained insoluble and was removed by filtration and washed with hexanes. The crude enolate was used without further purification. 1H NMR (DMSO-d_6,TSP): δ 8.46 (2 H, H2' and H6'), 7.63 (1 H, H4'), 7.35 (1 H, H5'), 3.14 (2 H, H6'), 2.25 (2 H, H4'), 1.60 (2 H, H5').

Lithium Enolate of 1-Methyl-3-nicotinoyl-2-piperidone (6-2) was prepared according to known procedures [97] using 6.0 mL (53 mmol) of 1-methyl-2-piperidone and 36 mL (54 mmol) of 1.5 M LDA in cyclohexane and 50 mL of THF and a solution of 3.7 mL (0.027 mmol) of ethyl nicotinate in 24 mL of THF. The lithium enolate precipitated from the reaction mixture and was removed by filtration and washed with hexanes.

^{1}H NMR (DMSO-d_6, TSP): δ 8.44 (m, 2 H, H2', H6'), 7.62 (d, 7.6 Hz, 1 H, H4'), 7.34 (dd, 7.6, 4.7 Hz, 1 H, H5'), 3.24 (t, 5.6 Hz, 2 H, H6), 2.88 (s, 3 H, NCH_3), 2.26 (broad, 2 H, H4), 1.66 (m, H5). ^{13}C NMR (DMSO-d_6, TSP): δ 174.8, 168.2, 148.0, 147.2, 141.5, 134.3, 122.8, 90.9, 49.6, 34.3, 28.2, 24.1.

Lithium Enolate of 1-Methyl-3-benzoyl-2-piperidone (6-3) was prepared as (6-2) using 12 mL (18 mmol) of 1.5 M LDA in cyclohexane and 2.1 mL (19 mmol) of 1-methyl-2-piperidone in 15 mL of THF and a solution of 1.23 g (9.0 mmol) of methyl benzoate in 6 mL of THF. After diluting the reaction mixture with hexanes, the lithium enolate was removed by filtration and used without further purification. ^{1}H NMR (DMSO-d_6, TSP): δ 7.31 (m, phenyl), 3.22 (t, H6), 2.87 (s, NCH_3), 2.26 (unresolved, H4), 1.64 (unresolved, H5).

3-Nicotinoyl-2-piperidone (6-7). Two preparations are reported giving products with different melting points. They are believed to contain different amounts of keto and enol material.

A suspension of 382 mg of the enolate 6-1 in water was neutralized by the addition of 2 M HCl until the pH was about 7-8 and the sample dissolved. Extraction of this solution with ethyl acetate, followed by evaporation afforded 272 mg of 3-nicotinoyl-2-piperidone (mp 114-122 °C, 74% yield based on condensation with ethyl nicotinate). A deep blue to purple colored solution is obtained on addition of product to an aqueous solution of Fe^{3+}. This represents a sensitive test for detection of the enolizable proton in the product [58].

A sample which was prepared 45 min before recording the ^1H NMR spectrum ($CDCl_3$) consisted of 58% enol and 42% keto tautomers. Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.87; H, 5.99; N, 13.76.

Another sample of the enolate 6-1 was neutralized and the resulting 3-nicotinoyl-2-piperidone was recrystallized from ethyl acetate (mp 126-131 °C) and again from ethanol (mp 127-129 °C). A ^1H NMR spectrum of this solid in $CDCl_3$ recorded 15 min after mixing consisted of the enol almost exclusively. After 22 hrs, the sample was composed of 71% enol and 29% keto and after 2 days the sample consisted of 61% enol and 39% keto. The ratio of enol to keto did not change further. ^1H NMR ($CDCl_3$, TMS), enol: δ 15.10 (s, 1 H, OH), 8.77 (s, 1 H, H2'), 8.63 (d, 1 H, H6'), 7.86 (dt, 7.9, 1.9 Hz, 1 H, H4'), 7.37 (dd, 7.8, 4.9 Hz, 1 H, H5'), 3.39 (t, 2 H, H6'), 2.54 (2 H, H4), 1.80 (quintet, 5.9 Hz, 2 H, H5). Keto: δ 9.22 (s, H2'), 8.8 (H6', overlaps H2' of enol), 8.32 (dt, 8.0, 1.9 Hz, H4'), 7.44 (dd, 8.0, 4.8 Hz, H5'), 4.38 (t, 6.8 Hz, H3), 3.4 (t, H6 overlaps enol H6), 2.25, 2.05(m, H4 and H5). ^{13}C NMR ($CDCl_3$), enol: δ 172.35, 165.32, 150.26, 149.24, 135.72, 131.33, 123.09, 97.84, 41.95, 24.85, 22.77. Anal. Calcd. for $C_{11}H_{12}N_2O_2$ (mp 130-133 °C): C, 64.69; H, 5.92; N, 13.72. Found: C, 64.51; H, 5.92; N, 13.71.

Anabaseine Dihydrochloride (3,4,5,6-Tetrahydro-2,3'-bipyridinium Dihydrochloride) (6-4.2HCl). Following hydrolysis and decarboxylation of 4.94 g of the lithium enolate 6-1 in 30 mL of concentrated HCl, the reaction

mixture was diluted to 350 mL with isopropyl alcohol. The dihydrochloride crystallized slowly to yield 3.88 g of white needle-like crystals (mp 173-178 °C, decomp). The product was recrystallized by suspending it in 200 mL of hot isopropyl alcohol and then adding dropwise sufficient 6 M HCl to effect solution to yield on cooling 3.26 g (13.0 mmol, 56% yield) of fine white needle-like crystals (mp 175-180 °C, decomp, dependent on rate of heating). The dipicrate has been prepared [59]. ^{1}H NMR (D_2O), ketone: δ 9.34 (s, H2'), 9.08 (dt, 8.3, 1.6 Hz, H4'), 8.99 (d, 5.9 Hz, H6'), 8.22 (dd, 8.1, 5.9 Hz, H5'), 3.30 (t, 6.6 Hz, H2), 3.07 (t, 6.6 Hz, H5), 1.81 (m, H3 and H4). Imine (7%): δ 9.04 (s, H2'), 8.92 (d, H6'), 8.45 (dt, H4'), 7.86 (dd, H5'), 3.91 (broad, H6), 2.04 (m, H4 and H5). Anal. Calcd. for $C_{10}H_{12}N_2.2HCl.H_2O$: C, 47.82; H, 6.42; N, 11.15. Found: C, 47.92; H, 6.55; N, 10.96.

Anabaseine Dihydrobromide (3,4,5,6-Tetrahydro-2,3'-bipyridinium Dihydrobromide) (6-4.2HBr). Following hydrolysis and decarboxylation of 0.58 g of crude 6-1 in 7 mL of conc. HBr, the solvent was evaporated to 3-4 mL of brown liquid, diluted with 15-20 mL of isopropyl alcohol, and stirred to yield 0.573 g of the dihydrobromide as a light brown precipitate (mp 166-170 °C, decomp). This solid was recrystallized from conc. HBr and ethanol to yield 0.430 g (1.26 mmol, 45% yield) of light brown needle-like crystals (mp 167-170 °C, decomp). An analytical sample was prepared by recrystallization from methanol and acetonitrile

(mp 168.5-172 °C, decomp). Anal. Calcd. for $C_{10}H_1N_2.2HBr.H_2O$: C, 35.32; H, 4.74; N, 8.24. Found: C, 35.49; H, 4.76; N, 8.20.

N-Methylanabaseine Chloride Hydrochloride (1-Methyl-3,4,5,6-tetrahydro-2,3'-bipyridinium Chloride Hydrochloride) (6-5.Cl.HCl). Following hydrolysis and decarboxylation of 3.02 g of lithium enolate 6-2 in 10 mL of H_2O and 50 mL of conc. HCl according to known procedures [97, 96], the HCl was evaporated to about 5 mL of brown liquid, dissolved in a minimum amount of ethanol, and diluted with acetonitrile to yield 2.13 g of the crude chloride hydrochloride as a white precipitate. The solid was recrystallized from ethanol and acetonitrile to yield 1.47 g (5.54 mmol) of white solid (mp 175.5-177 °C, decomp). The mother liquor was evaporated and the solid residue was recrystallized from ethanol and acetonitrile to yield 0.38 g (1.43 mmol) of a second crop of white solid (mp 171-174 °C, decomp, 61% combined yield). An analytical sample was prepared by two further recrystallizations from ethanol and acetonitrile (173-177 °C, decomp). The monopicrate has been prepared [58]. ^1H NMR (0.5 M NaCl, D_2O), amino ketone: δ 9.34 (d, 1.6 Hz, 1 H, H2'), 9.07 (dt, 8.1, 1.7 Hz, 1 H, H4'), 9.00 (d, 5.8 Hz, 1 H, H6'), 8.21 (dd, 8.2, 5.7 Hz, 1 H, H5'), 3.32 (t, 2 H, H2), 3.13 (unresolved t, H5), 2.75 (s, NCH_3), 1.84 (m, 2 H, H3 and H4). ^{13}C NMR (0.5 M NaCl, D_2O), amino ketone: δ 201.31, 147.90, 147.71, 145.15, 137.52, 130.40, 51.69, 41.04, 35.67, 27.61, 22.46. Anal. Calcd. for $C_{11}H_{15}N_2.HCl.Cl.H_2O$:

C, 49.82; H, 6.84; N, 10.56. Found: C, 49.83; H, 6.88; N, 10.51.

1-Methyl-2-phenyl-3,4,5,6-tetrahydropyridinium Bromide (6-6.Br). Following hydrolysis and decarboxylation of 1.03 g of the lithium enolate 6-3 in 20 mL of conc. HBr, the reaction mixture was evaporated to a brown oil and diluted with acetone. On standing overnight 60 mg (0.22 mmol, 5% yield) of cream colored flaky crystals formed (mp 143-146° C). ^{1}H NMR (D_2O), ketone: δ 8.01 (d, phenyl, ortho), 7.63 (m, phenyl, overlaps imine), 3.19 (t, H2), 3.10 (t, H5), 2.74 (s, NCH_3), 1.78 (m, H3 and H4). Imine: δ 7.63 (m, phenyl, overlaps ketone), 3.96 (t, H6), 3.49 (s, NCH_3), 2.09, 1.97 (quintets, H4 and H5). The picrate has been prepared [58]. ^{1}H NMR of the perchlorate in trfluoroacetic acid solution has been reported [126].

Anabaseine Hydrochloride (6-4.HCl). To a solution of 98.2 mg of 6-4.2HCl in 3 mL of methanol was added 166 mg of poly-4-vinylpyridine. After stirring the suspension at room temperature for 2 hrs, the poly-4-vinylpyridine was removed by filtration. The methanolic filtrate was evaporated to a light brown solid (mp 118.5-120 °C, decomp). ^{1}H NMR (D_2O, TSP), ketone: δ 9.09 (d, 1.4 Hz, H2'), 8.75 (dd, 4.9, 1.4 Hz, H6'), 8.40 (dt, 8.0, 1.7 Hz, H4'), 7.63 (dd, 8.0, 4.9 Hz, H5'), 3.23 (t, residual H2), 3.08 (t, H5), 1.79 (m, H3 and H4). Imine: δ 8.95 (d, 1.9 Hz, H2'), 8.85 (dd, 5.0, 1.4 Hz, H6'), 8.29 (dt, 8.3, 1.7 Hz, H4'), 7.72 (dd 8.1, 5.0 Hz H5'),

3.91 (broad, H6), 2.04 (broad, H4 and H5). Anal. Calcd. for $C_{10}H_{12}N_2.HCl.H_2O$: C, 55.95; H, 7.04; N, 13.05. Found: C, 55.93; H, 7.02; N, 12.83.

Anabaseine Hydrobromide (6-4.HBr) was prepared from the dihydrobromide by the method reported for 6-4.HCl (mp 88-90 °C). Anal. Calcd. for $C_{10}H_{12}N_2.HBr.H_2O$: C, 46.35; H, 5.83; N, 10.81. Found: C, 46.72; H, 5.67; N, 10.60.

N-Methylanabaseine Chloride (6-5.Cl). To a solution of 108 mg (0.407 mmol) of 6-5.Cl.HCl in 2 mL of methanol was added 175 mg of poly-4-vinylpyridine. After stirring the suspension for 2 hrs at room temperature, the poly-4-vinylpyridine was removed by filtration. The methanol was evaporated to 88 mg (0.38 mmol, 93% yield) of white solid (mp 135.5-138 °C, decomp). ^1H NMR (D_2O, TSP), amino ketone: δ 9.09 (d, 2.3 Hz, H2'), 8.74 (dd, 5.0, 1.7 Hz, H6'), 8.39 (dt, 8.1, 2.0 Hz, H4'), 7.61 (dd, 8.1, 5.0 Hz, H5'), 3.23 (t, 6.4 Hz, H2), 3.12 (t, 6.9 Hz, H5), 2.76 (s, NCH_3), 1.81 (m, H3 and H4). Imine: δ 8.81 (dd H6'), 8.72 (d, H2'), 8.06 (dt, H4'), 7.72 (dd, H5'), 4.03 (t, H6), 3.56 (s, NCH_3), 2.13, 2.00 (quintets, H4 and H5). Anal. Calcd. for $C_{11}H_{15}N_2Cl.H_2O$: C, 57.76; H, 7.49; N, 12.25. Found: C, 57.63; H, 7.40; N,12.08.

Ethyl 5-(N,N-Dimethylamino)pentanoate (6-8). To a solution of 3.38 g (22.5 mmol) of sodium iodide in 40 mL of ethanol was added 1.00 g (12.3 mmol) of dimethylamine hydrochloride. After stirring the mixture at room temperature for 10 min, the solid (NaCl) was removed by

filtration. To the remaining ethanolic solution was added 2.10 g (10.0 mmol) of ethyl 5-bromopentanoate and 4.02 (37.9 mmol) of sodium carbonate. After heating at reflux for 3 1/2 hours and cooling, the insoluble salts were filtered out and the ethanol filtrate was evaporated to an oil mixed with solid. Solid salts were removed by filtration after dissolving the residue in 3/2 ethyl acetate / hexanes. After evaporation of the solvent, the residue was partially dissolved in methylene chloride and insoluble material was removed. The methylene chloride was evaporated to 1.66 g of a liquid residue which consisted of 2 phases. The liquid residue was partially dissolved in 30 mL of an aqueous solution of sodium carbonate and sodium chloride (pH ≈ 10). The aqueous phase was extracted with 3 25-mL portions of chloroform and the chloroform extract was evaporated to a liquid residue. The residue was stirred in 10 - 15 mL of cyclohexane overnight before separating the cyclohexane from an insoluble oil. The insoluble oil consisted of 0.527 g (1.74 mmol, 17% yield) of the diester resulting from dialkylation of dimethylamine. The cyclohexane solution was evaporated to 0.846 g (4.88 mmol, 49 % yield) of volatile yellow liquid. ^{1}H NMR ($CDCl_3$, TMS) δ 4.128 (ethoxy CH_2, 2 H, q, J = 7.1 Hz), 2.323 (H2, part of 9 H, t, J = 7.4 Hz), 2.263 (H5, part of 9 H, m, not 1st order), 2.210 ($N(CH_3)_2$, part of 9 H, s), 1.60-1.71 (H3 or 4, 2 H, m), 1.44-1.55 (H3 or 4, 2 H, m), 1.254 (ethoxy CH_3, 3 H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 173.38, 60.01, 59.21, 45.31, 34.05, 27.04, 22.72, 14.09 .

Ethyl 5-(N,N-dimethylamino)-2-nicotinoylpentanoate (6-9). To a flame dried flask charged with nitrogen and 7 mL of dry THF at -70 °C was added 6.1 mL (9.2 mmol) of a solution of 1.5 M lithium diisopropylamide in cyclohexane. The solution was cooled in a dry ice/acetone bath for 30 min before adding a solution of 0.791 g (4.56 mmol) of ethyl 5-(N,N-dimethylamino)pentanoate in 4 mL of THF. After stirring the reaction mixture at -70 °C for 1 hr, a solution of 0.691 g (4.57 mmol) of ethyl nicotinate in 3 mL of THF was added. The reaction mixture was stirred at -70 °C for 3 1/2 hrs and at room temperature for 20 hrs. A solution of 2 M HCl was added (10-12 mL) until the pH of the aqueous phase was about 6. The organic phase was diluted with ethyl acetate before separating the phases. The aqueous phase was washed with 25 mL of ethyl acetate. Solid sodium carbonate was added to the aqueous phase until pH ≈ 9-10 and the basic solution was extrated with 4 25-mL portions of ethyl acetate. The combined ethyl acetate phases were dried over sodium sulfate and evaporated to yield 0.789 g (2.83 mmol, 62% crude yield) of a rust colored oil. The oil was used without further purification.

5-(N,N-Dimethylammonio)-1-(3-pyridinio)pentanone Dihydrochloride (6-10). A solution of 0.789 g (2.83 mmol) of 6-9 in 6 mL of 16% aqueous HBr was heated at 100 °C for 4 1/2 hrs. The bromide salt could not be easily purified. The solution was diluted with 20 mL of water and washed with 20 mL of ethyl acetate. Solid sodium carbonate was added to the

aqueous solution until the pH was about 10. The basic solution was extracted with 3 20-mL portions of chloroform and the combined chloroform layers were washed with brine. The brown chloroform solution was treated with charcoal to give a yellow solution. The chloroform was evaporated to 0.487 g of dark liquid which contained predominantly hydrolyzed and decarboxylated product. A solution of the liquid in ethanol was acidified by adding 20 drops of concentrated HCl. The solvent was evaporated to a brown liquid which was stirred with isopropyl alcohol to produce 0.317 g (1.14 mmol, 40% yield) of light beige solid precipitate (mp 150-156 °C, decomp). Recrystallization of the solid from a solution of isopropyl alcohol containing a drop of concentrated HCl yielded 0.240 g (0.807 mmol, 29% yield) of light brown solid hydrate (mp 134.5-136 °C). An analytical sample was prepared by recrystallization twice from ethanol containing a drop of conc. HCl and ethyl acetate (mp 136-138 °C). ^{1}H NMR (D_2O, TSP) δ 9.313 (H2', 1 H, s), 9.040 (H4', 1 H, d, $J_{4,5}$ = 8.2 Hz), 8.971 (H6', 1 H, d, $J_{5,6}$ = 5.8 Hz), 8.184 (H5', 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 5.8 Hz), 3.295 (H2, 2 H, t, J = 6.4 Hz), 3.211 (H5, 2 H, m, not 1st order), 2.900 ($N(CH_3)_2$, 6 H, s), 1.78-1.90 (H3 and H4, 4 H, m). ^{13}C NMR (D_2O, TSP) δ 201.79, 148.97, 146.69, 145.99, 137.27, 130.05, 60.41, 45.62, 40.96, 26.27, 22.48. Anal. Calcd. for $C_{12}H_{18}N_2O.2HCl.H_2O$: C, 48.49; H, 7.46; N, 9.42. Found: C, 48.15; H, 7.51; N, 9.26.

(S)-N-Methylanabasine Dihydrobromide (6-11) was prepared according to known procedure [99] using a solution of 500 μL (6.4 mmol) of 37% aqueous formaldehyde, 0.75 mL (17 mmol) of 90% formic acid, and 0.253 g (1.56 mmol) of (S)-anabasine and heating at 100 °C for 7 1/2 hrs. The reaction mixture was diluted with 4 mL of H_2O and solid sodium carbonate was added until the solution was basic. The solution was extracted with 3 10-mL portions of ether. The ether phase was dried over $MgSO_4$ and evaporated to give 0.239 g (1.36 mmol, 87% yield) of yellow liquid. ^1H NMR ($CDCl_3$, TMS) δ 8.44-8.55 (H2' and H6', 2 H, m), 7.70 (H4', 1 H, d), 7.27 (H5', 1 H, dd), 3.04 (H2 or H6, 1 H, d), 2.83 (H2 or H6, 1 H, dd), 2.13 (H2 or H6, 1 H, m), 2.00 (NCH_3, 3 H, s), d at 1.83, m at 1.72, qd at 1.57, and m at 1.39 (H3, H4, and H5, 6 H).

The dihydrobromide salt was prepared by adding several drops of conc. HBr to a sample of the free base in ethyl acetate. When two phases formed, enough isopropyl alcohol was added to make the phases miscible and an off-white solid precipitated (mp 217-220 °C, decomp). Recrystallization was accomplished by adding methanol to a boiling suspension of 119 mg of the salt in isopropyl alcohol. On cooling, 101 mg of white solid was collected. ^1H NMR (D_2O, TSP) δ 8.989 (H2', 1 H, s), 8.920 (H6', 1 H, d, $J_{5,6}$ = 5.6 Hz), 8.740 (H4', 1 H, d, $J_{4,5}$ = 8.1 Hz), 8.194 (H5', 1 H, dd, $J_{4,5}$ = 8.3 Hz, $J_{5,6}$ = 5.9 Hz), 4.542 (H2 or H6, 1 H, dd, J = 11.3, 4.1 Hz), 3.753 (H2 or H6, 1 H, d with unresolved minor coupling, J = 13.7 Hz), 3.310 (H2 or H6, 1 H, td, J = 12.8, 3.3 Hz), 2.679

(NCH_3, 3 H, s), 1.65 -2.25 (H3, H4, and H5, 6 H, m). ^{13}C NMR (D_2O, TSP) δ 147.68, 146.92, 145.52, 137.58, 131.00, 69.38, 60.14, 44.44, 34.11, 26.02, 24.72. Anal. Calcd. for $C_{11}H_{16}N_2.2HBr.1/2H_2O$: C, 38.07; H, 5.52; N, 8.07. Found: C, 38.25; H, 5.42; N, 7.84.

General Palladium Catalyzed Coupling Procedure. 6-Methyl-2,3'-bipyridine (6-12). To a flask charged with nitrogen was added 0.808 g (5.50 mmol) of diethyl(3-pyridyl)borane, 0.846 g (6.63 mmol) of 2-chloro-6-methylpyridine, 0.683 g (0.591 mmol) of tetrakis(triphenyl)phosphine palladium (0), and 25 mL of THF which was degassed by bubbling nitrogen through it for 10-15 min. After stirring this mixture for 10-15 min, the solids dissolved and a degassed solution of 1.61 g (11.6 mmol, 2.1) of K_2CO_3 in 13 mL of water was added. The reaction mixture was heated at reflux for 6 hrs with vigorous stirring. The THF layer went from brown to yellow during reflux. The palladium catalyst is not sensitive to water but is sensitive to air especially when in a warm solution. The progress of the reaction was monitored by TLC on silica gel using a 1:12 acetone/hexanes eluent or a 1:7 ethyl acetate/hexanes eluent. On cooling the reaction mixture under nitrogen, the Pd catalyst, a bright yellow solid, crystallized and was filtered under nitrogen (mp 103-109°C, decomp, lit. mp 115 °C under N_2 [127]). The filtrate was then diluted with 50 mL of ethyl acetate and the phases were separated. The organic phase was washed with a 13-mL portion of brine and the

combined aqueous phases were washed with a 10-mL portion of ethyl acetate. The combined organic layers were extracted first with a 10-mL portion of 2 M HCl and then with a 5-mL portion of HCl. The combined HCl layers were made slightly basic by the addition of solid Na_2CO_3 (pH ≈ 8-9). This basic solution was then extracted with 4 10-mL portions of methylene chloride. The methylene chloride solution was dried over magnesium sulfate before evaporating to 0.88 g of a yellow oil (94% crude yield). The coupled products are reasonably pure after the acid workup. The oil was purified by flash column chromatography on silica gel with an ethyl acetate eluent to yield 0.747 g of clear liquid (85% yield). ^{1}H NMR ($CDCl_3$, TMS) δ 9.173 (H2', 1 H, dd, $J_{2,4}$ = 2.3 Hz, $J_{2,5}$ = 0.9 Hz), 8.634 (H6', 1 H, dd, $J_{5,6}$ = 4.8 Hz, $J_{4,6}$ = 1.7 Hz), 8.314 (H4', 1 H, ddd, $J_{4,5}$ = 8.0 Hz, $J_{2,6}$ = 2.3 Hz, $J_{4,6}$ =1.7 Hz), 7.672 (H4, 1 H, t, $J_{3,4} \approx J_{4,5}$ = 7.7 Hz), 7.538 (H3, 1 H, d, $J_{3,4}$ = 7.8 Hz), 7.387 (H5', 1 H, ddd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.8 Hz, $J_{2,5}$ = 0.9 Hz), 7.152 (H5, 1 H, d, $J_{4,5}$ = 7.6 Hz), 2.633 (NCH_3, 3 H, s). ^{13}C NMR ($CDCl_3$, TMS) δ 158.87, 154.16, 149.65, 148.30, 137.09, 135.17, 134.40, 123.51, 122.36, 117.63, 24.68.

A solution of 199 mg (1.17 mmol) of the free base in 2 mL of ethanol was converted to the dihydroperchlorate by adding 70% perchloric acid dropwise while in an ice bath. The diperchlorate precipitated to yield 0.360 g of white solid (mp 204-205 °C). Recrystallization from ethanol 3 times yielded 0.297 g (0.800 mmol) of white crystals (mp 202-

205°C). Anal. Calcd. for $C_{11}H_{10}N_2.2HClO_4$: C, 35.60; H, 3.26; N, 7.55. Found: C, 35.66; H, 3.20; N, 7.47.

2-(3-Pyridyl)pyrimidine (6-13) was prepared by the method described for 6-methyl-2,3'-bipyidine using 0.265 g (1.80 mmol) of diethyl(3-pyridyl)borane, 0.248 g (2.17 mmol) of 2-chloropyrimidine, and 0.148 g (0.13 mmol) of tetrakis(triphenylphosphine) palladium (0) in 8 mL of THF and a solution of 0.520 g (3.76 mmol) of potassium carbonate in water. After heating at reflux for 20 hrs, 0.204 g of yellow solid was obtained on work up (72% crude yield). Recrystallization of the solid from ethyl acetate and hexanes gave 65 mg of off-white needle-like crystals (mp 50-52°C, 23% yield). Preparation of this compound by Pd (0) catalyzed coupling was reported previously [17]. ^1H NMR ($CDCl_3$, TMS) δ 9.652 (H2', 1 H, dd, $J_{2,4}$ = 2.2 Hz, $J_{2,4}$ = 0.9 Hz), 8.843 (H4 and H6, 2 H, d, $J_{4,5} = J_{5,6}$ = 4.9 Hz), 8.68-8.74 (H4' and H6', 2 H, m), 7.432 (H5', 1 H, ddd, $J_{4,5}$ = 7.9 Hz, $J_{5,6}$ = 4.9 Hz, $J_{2,5}$ = 0.9 Hz), 7.267 (H5, 1 H, t, $J_{4,5} = J_{5,6}$ = 4.9 Hz). ^{13}C NMR($CDCl_3$, TMS) δ 163.00, 157.40, 151.38, 149.83, 135.47, 133.10, 123.42, 119.78. Anal. Calcd. for $C_9H_7N_3$: C, 68.78; H, 4.49; N, 26.73. Found: C, 68.53; H, 4.45; N, 26.61.

5-Methyl-2,3'-bipyridine (6-14) was prepared according to the procedure described for 6-methyl-2,3'-bipyridine using 0.152 g (1.04 mmol) of diethyl(3-pyridyl)borane, 0.208 g (1.21 mmol) of 2-bromo-5-methylpyridine, and 0.189 g (0.16 mmol) of tetrakis(triphenylphosphine) palladium (0) in 6 mL of THF and a solution of 0.425 g (3.08 mmol) of potassium

carbonate in 3mL of water. After heating at reflux for 6 hrs and work up, 0.150 g of yellow liquid was collected (85% crude yield). Another synthesis has been reported [128]. ^{1}H NMR ($CDCl_3$, TMS) δ 9.163 (H2', 1 H, unresolved dd), 8.624 (H6', 1 H, dd, $J_{5,6}$ = 4.8 Hz, $J_{4,6}$ = 1.7 Hz), 8.548 (H6, 1 H, s with unresolved minor coupling), 8.306 (H4', 1 H, ddd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 1.7 Hz, $J_{2,4}$ = 2.3 Hz), 7.58-7.69 (H3 and H4, 2 H, m), 7.401 (H5', 1 H, ddd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.8 Hz, $J_{2,5}$ = 0.9 Hz), 2.390 (NCH_3, 3 H, s). ^{13}C NMR ($CDCl_3$, TMS) δ 151.96, 150.50, 149.34, 147.85, 137.54, 134.95, 134.28, 132.63, 123.63, 120.12, 18.22.

3-Chloro-2,3'-bipyridine (6-15) was prepared according to the method described for 6-methyl-2,3'-bipyridine using 0.254 g (1.73mmol) of diethyl(3-pyridyl)borane, 0.303 g (2.05 mmol) of 2,3-dichloropyridine, and 0.210 g (0.18 mmol) of tetrakis(triphenylphosphine) palladium (0) in 8 mL of THF and a solution of 0.711g (5.14 mmol) of potassium carbonate in 4 mL of water. After heating at reflux for 24 hrs and extraction work up, a solid was obtained. This solid was recrystallized from ethyl acetate and hexanes to give 52 mg off-white needle-like crystals (mp 66.5-68.5 °C). A second crop was collected to yield 29 mg of white crystals (mp 66-67.5 °C, 30% total yield). ^{1}H NMR δ 9.013 (H2', 1 H, dd, $J_{2,4}$ = 2.3 Hz, $J_{2,5}$ = 0.8 Hz), 8.678 (H6', 1 H, dd, $J_{5,6}$ = 4.9 Hz, $J_{4,6}$ = 1.7 Hz), 8.632 (H6, 1 H, dd, $J_{5,6}$ = 4.6 Hz, $J_{4,6}$ = 1.5 Hz), 8.078 (H4', 1 H, dt, $J_{4,5}$ = 7.9 Hz, $J_{2,4} \approx J_{4,6}$ = 2 Hz), 7.840 (H4, 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{4,6}$ = 1.5 Hz), 7.414 (H5',

1 H, ddd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.9 Hz, $J_{2,5}$ = 0.8 Hz), 7.290 (H5, 1 H, dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 4.9 Hz). ^{13}C NMR($CDCl_3$) δ 153.66 (C2), 150.24, 149.70, 147.95, 138.24, 136.77, 133.99, 130.51, 123.76, 122.84.

1-Methyl-4,4'-bipyridinium Iodide (7-1). A solution of 0.500 g (3.20 mmol) of 4,4'-bipyridine and 0.50 mL (8.0 mmol) of methyl iodide in 1/4 cyclohexane/ethyl acetate was stirred at room temperature for 20 hrs. The resulting precipitate was removed by filtration to yield 0.450 g of bright yellow solid (mp 242-243 °C, decomp, 47% crude yield, lit. mp 244 °C [129]). Recrystallization from ethanol 3 times with hot filtration to remove a darker orange material yielded 0.190 g of fine yellow crystals (mp 242-244 °C, decomp). An analytical sample was prepared by a fourth recrystallization from ethanol (mp 245-246 °C, decomp). ^1H NMR (D_2O, TSP) δ 8.925 (H2, 2 H, d, $J_{2,3}$ = 7.0 Hz), 8.792 (H2', 2 H, unsymmetric dd), 8.408 (H3, 2 H, d, $J_{2,3}$ = 6.8 Hz), 7.932 (H3', 2 H, unsymmetric dd), 4.464 (NCH_3, 3 H, s). Anal. Calcd. for $C_{11}H_{11}N_2I$: C, 44.52; H, 3.72; N, 9.40. Found: C, 44.29; H,3.62; N, 9.25.

Measurements and Calculations

Measurement of Equilibrium Compositions of Aqueous Anabaseine Solutions

Anabaseine $2HClO_4 \cdot 1/2H_2O$ (mp 106-109° C) was used for these measurements. Anal. Calcd for $C_{10}H_{15}N_2Cl_2O_{8.5}$:

C, 32.45; H, 4.08; N, 7.57. Found: C, 32.67; H, 4.29; N, 7.45. This material was prepared by Dr. William R. Kem and isolated as the picrate as originally reported [59] and then converted to the perchlorate.

Buffers

Solutions for NMR measurements were prepared by dissolving the buffer salts in a solution of 0.5 M NaCl and 0.002 M TSP (sodium 3-(trimethylsilyl)propionate) in 99.8% D_2O. Phosphate and carbonate buffers ranged in ionic strength from 0.52 M to 0.68 M. Measurements of pH were made using Bates [125] buffers for standardization. The pH-meter readings were converted to pD by adding 0.40 [130].

Solutions for UV measurements were prepared by making 0.01 M solutions of the acidic buffer component and adding KCl to make an ionic strength of 0.15 M. Acidic buffer was mixed with an appropriate volume of 0.01 M NaOH and 0.14 M KCl solution to bring the buffer to the desired pH. Glass distilled water was used. Buffers include formate, acetate, MES, HEPES, bicine, and carbonate ion.

^{1}H NMR measurements

Spectra were recorded at ambient temperature (usually 21-23 ^{o}C). Samples were prepared by dissolving 2-4 mg of anabaseine in 0.4-0.6 mL of the appropriate buffer; concentrations ranged from 0.008 M to 0.018 M. A pD value was obtained by measuring the pH and adding 0.40 for each sample after recording the spectrum. The ratios of the areas

of the methylene protons α to the nitrogen atom in the open chain form and the methylene protons α to the imine nitrogen in the cyclic form were obtained by integration. A delay time of 5 s between transients was used. This is 2.9 and 3.3 times the two T_1's of interest. Using a delay of 10 s which is 5.7 and 6.5 times the two T_1's of interest did not have a significant effect (±2%) on the integral ratios so the shorter delay was used. Each spectrum was the result of 64 transients. The methyl signal of TSP served as an internal shift standard.

UV measurements

A 3-mL aliquot of each buffer was equilibrated in the spectrophotometer at 25° C for 15 min before adding 100 μL of 3.57 mM anabaseine in 0.15 M KCl. A wavelength scan from 220 to 320 nm was recorded for each sample. The following molar absorbtivities were used with eq 3: 2-1, 5570; 2-2, 10,000 - 10,500; 2-3, 5,000 - 5,200; 2-4, 1790.

Computer program

The program written by Dr. D. Whitman does a non-linear least squares analysis to fit parameters to observed data and the equations given in the text. It is based on the program of Wentworth [131].

Measurement of the Compositions of Solutions of Anabaseine and N-Methylanabaseine in Nonaqueous Solvent Systems

^{1}H NMR measurements

NMR samples were prepared by weighing the compound into an NMR tube and adding 500 mL of solvent. Concentrations ranged from 0.04 to 0.16 M. The spectra were recorded at probe temperature (21-23 °C).

Spectra were recorded using a 37° pulse and no delay between transients. The fraction of a given component was obtained by averaging the values calculated using the intensity ratios for several sets of peaks. Spectra recorded using a 37° pulse and with no delay between transients gave similar intensity ratios to those recorded using a 90° pulse and a delay of 5 times the longest T_1. A ^{1}H NMR spectrum of 4-3.Cl in CD_3OD gave values of 71.8% imine, 22.4% ketone, and 5.78% enamine when recorded with no delay between transients and gave values of 72.0% imine, 22.2% ketone, and 5.80% enamine when recorded with a delay time of 35 s ($5xT_1$) between transients.

Chemical shifts for compounds 4-1 and 4-3 in nonaqueous solvents appear in Tables 4-2 and 4-3. Their chemical shifts and multiplicities are similar to those reported for the aqueous solutions in the preparations. Results are reported in Table 4-1.

Control experiments to establish that the hydrolysis reaction is at its equilibrium position

No change in the composition of 4-3 in D_2O was observed for a sample at pD 6.0 after three days at room temperature, showing that the initial spectrum obtained after a few minutes represented the sample at equilibrium as expected.

All samples but 4-3.Cl.HCl in DMSO were checked, usually after several days. No change was detected except in the case of 4-1.HBr where a 40 minute-old sample gave 35% ketone and 65% imine but 9 days later showed 15% ketone and 85% imine. The latter is taken to be the equilibrium composition, Table 4-1.

With the exception of 4-3.Cl.HCl which was not checked for time dependence in methanol, the data reported in Table 4-1 represent stable samples. When 4-1.2HBr was examined after standing 10 days at room temperature a substantial decrease in the amount of ketone and an increase in the amount of imine and unknown was noted. The initial reading was taken to be that for the equilibrium mixture.

Titration of methanolic solutions of anabaseine.HCl and N-methylanabaseine.Cl with D_2O

Initial samples were prepared by weighing the compounds into NMR tubes and adding 500 mL of CD_3OD. ^1H NMR spectra were recorded a few minutes after preparation. Both samples then were given several hours to come to equilibrium before titration. After 21 hrs, the 0.080 M solution of 4-1 went from 92% to 96% imine and after 8 hrs, the 0.079 M solution

of 4-3 went from 74% to 76% imine. The latter values were taken as the equilibrium values. Seven to eight aliquots of D_2O ranging in size from 15-60 μL were added successively to the samples 10 min before recording the spectra. After 3 days, the solution of 4-1 containing 150 μL of D_2O and 500 mL of CD_3OD had not changed significantly (47.6% to 47.4% imine). A final aliquot of 60 μL of D_2O was added to this solution.

Prior to fitting the titration data by computer, eq 4-3 was transformed into eq 8-2 to calculate the water impurity in the CD_3OD, considering this amount as an adjustable parameter. The ratio, K_D/K_M, and K_M were also fit as adjustable parameters.

$$\ln K_{mix} = \ln \left(\frac{[AK]}{[Im][D_2O]_{tot}} \right) = \ln \left[\left(\frac{K_D}{K_M} \right)^{F_D} \cdot K_M \right] \qquad (8\text{-}1)$$

$$\ln \frac{[AK]}{[Im]} = \ln \left[\left(\frac{K_D}{K_M} \right)^{F_D} \cdot K_M \cdot [D_2O]_{tot} \right] \qquad (8\text{-}2)$$

where [AK] = concentration of amino ketone and

[Im] = concentration of imine.

The mole fraction of water, F_D, and the concentration of water were expressed in terms of moles of water, eqs 8-3 and 8-4.

$$F_D = \frac{M_{sub} + M_{solv} + M_o}{M_{sub} + M_{solv} + M_o + 0.0246(0.500 - 18.13M_o)} \quad (8\text{-}3)$$

$$[D_2O]_{tot} = \frac{M_{sub} + M_{solv} + M_o}{(0.01813)(M_{sub} + M_{solv}) + 0.000500} \quad (8\text{-}4)$$

where M_{sub} = moles of water liberated from substrate,

M_{solv} = moles of water from added D_2O, and

M_o = moles of water impurity in CD_3OD.

In eq 8-3, the volume of methanol (0.500 mL) is corrected for the volume of water impurity which is given by the term 18.13Mo where 18.13 is the ratio of the molecular weight (20.03 g/mol) to the density (1.105 g/mL) of D_2O [132]. The corrected volume of methanol is then converted to moles of CD_3OD by multiplying by 0.0246 which is the ratio of the density (0.888 g/mL) to the molecular weight (36.07 g/mol). In eq 8-4, the term 0.01813 is a conversion factor (the ratio of molecular weight, 20.03 g/mol, to density in grams per liter, 1105 g/L) for converting the moles of D_2O to liters and the term 0.000500 is the volume of methanol in liters. The moles of water liberated from cyclization of amino ketone, M_{sub}, was calculated for each ratio of amino ketone to cyclic imine measured. The fraction of imine ([Im]/([Im]+[AK])) present is equal to the fraction of water liberated. The moles of water liberated then is the product of the fraction of water and the total moles of substrate present. The value of the moles of water impurity in the methanol which was calculated with the computer fit

was added to the observed mole fraction of water, eq 8-3, and to the observed concentration of water to correct the experimental values of K_{mix}. The values for K_{mix} were plotted against the mole fractions of water in the LFER. Results appear in Figures 4-1 - 4-4.

Control experiments to measure the equilibrium constant for N-methylanabaseine.Cl in dry methanol

Measurements were made on two separate solutions of 4-3.Cl in methanol which was dried over 3A sieves prior to making the NMR solutions.

The 3A sieves were rinsed with D_2O and dried by heating them under vacuum for 10 hrs. For the first NMR solution, a sample of perdeuterated methanol was dried over these sieves for 22 hrs in an oven dried volumetric flask. Solid 4-3.Cl was dried in a drying pistol, weighed into an NMR tube containing dry TSP, and dissolved in 500 μL of the dried methanol to make a 0.078 M solution of 4-3.Cl. Three and a half hours after making the NMR sample, 72.9% imine, 22.9% amino ketone, and 4.2% enamine were present. After standing 32 hours, 71.7% imine, 25.7% amino ketone, and 2.5% enamine were present and after 3 days, 70.8% imine, 29.2% amino ketone, and a trace of enamine were present. It is not clear whether the increase in the fraction of amino ketone and decrease in the fraction of enamine represent a slow approach to equilibrium or contamination of the sample with water on standing. The values calculated for K_M (K_M =

[ketone]/([imine][D_2O]$_{tot}$)) are 5.2 M^{-1} after 3.5 hrs, 6.2 M^{-1} after 32 hrs, and 7.5 M^{-1} after 3 days.

The 3A molecular sieves were dried an additional 9 hrs for the second NMR sample. A fresh sample of perdeuterated methanol was dried over the sieves for 3 days in an oven-dried volumetric flask in a dessicator. Solid 4-3.Cl was dried in a drying pistol and weighed into an NMR tube with dry TSP. The NMR tube containing the solids was dried in the drying pistol before adding 500 μL of the dried CD_3OD with a dry syringe to make a 0.111 M solution of 4-3.Cl. This NMR sample was stored in a dessicator. After standing 22 hrs, the sample contained 72.0% imine, 22.2% ketone, and 5.8% enamine. After standing 46 hrs, the sample contained 72.0% imine, 22.1% ketone, and 5.9% enamine. The fractions of each component in the sample represent equilibrium values and yield a calculated value for K_M of 3.6 M^{-1} which is in agreement with the value obtained from the LFER fit (K_M = 3.3 M^{-1}) of 4-3.Cl in methanol which was not dried.

Reactions of 1,1'-Disubstituted-2,3'-bipyridines with Base

UV Measurements. A suitable amount of each bipyridinium compound was dissolved in methanol to give reasonable absorbances. Absorbances were recorded over a range of 200 to 500 nm or 220 to 500 nm. In samples where 2,2,6,6-tetramethylpiperidine was added, the piperidine absorbed below 240 nm.

Methoxide adduct of 1,1'-dimethyl-2,3'-bipyridinium diiodide in 4/1 DMSO-d_6/methanol-d_4

Standard ^1H NMR spectra were recorded by collecting 16 transients with no delay time between transients. TSP was used as an internal reference.

An NMR sample was prepared by weighing 22.0 mg (0.0500 mmol) of 5-3 into and NMR tube and dissolving the solid in 0.40 mL of DMSO-d_6 and 100 μL of methanol-d_4. After recording the proton spectrum of the starting material, 16.9 μL (0.100 mmol) of 2,2,6,6-tetramethylpiperidine was added. Proton NMR spectra were recorded 15 min and 1 3/4 hrs after the addition of the piperdine. Spectra recorded after the addition of base showed a mixture of 5-3 and a σ adduct. No change occurred in the spectrum on standing. A proton spectrum was then recorded 10 min after the addition of 13.7 μL (0.1 mmol) of 20% DCl in D_2O. Addition of DCl almost completely regenerated 1,1-dimethyl-2,3'-bipyridinium starting material with only a trace of the σ adduct present.

^1H NMR of 1,1'-Dimethyl-2,3'-bipyridinium Diiodide (5-3) Before the Addition of 2,2,6,6-Tetramethylpiperidine.

δ 9.479 (H2', s), 9.345 (H6 or H6', d, $J_{5,6}$ = 6.1 Hz), 9.308 (H6 or H6', d, $J_{5,6}$ = 6.2 Hz), 8.990 (H4', d, $J_{4,5}$ = 8.1 Hz), 8.855 (H4, t, $J_{3,4} = J_{4,5}$ = 7.9 Hz), 8.459 (H5 or H5', unresolved dd), 8.384 (H5 or H5', unresolved dd), 8.333 (H3, d, $J_{3,4}$ = 7.9 Hz), 4.510 (1'-NCH_3, s), 4.307 (1-NCH_3, s). A trace of adduct 5-11 is visible in the baseline even before the addition of base.

^{1}H NMR of 5-3 1 3/4 hrs After the Addition of 2,2,6,6-Tetramethylpiperidine. δ 9.502 (H2', s, 73% D incorporation compared to H4' intensity), 9.364 (H6 or H6', d, $J_{5,6}$ = 6.2 Hz), 9.324 (H6 or H6', d, $J_{5,6}$ = 6.1 Hz), 9.010 (H4', d, $J_{4,5}$ = 8.1 Hz), 8.869 (H4, t, $J_{3,4}$ = $J_{4,5}$ = 7.8 Hz), 8.472 (H5 or H5', unresolved dd), 8.30-8.43 (H5 or H5' and H3 overlapping H5 of 5-11), 4.53 (1'-NCH_3, s), 4.326 (1-NCH_3, s).

^{1}H NMR of Adduct 5-11 1 3/4 hrs After Addition of 2,2,6,6-Tetramethylpiperidine. δ 8.800 (H6, d, $J_{5,6}$ = 6.1 Hz), 8.30-8.43 (H5 overlaps H3 and H5 or H5' of 5-3), 7.948 (H3, d, $J_{3,4}$ = 8.3 Hz), 7.738 (H4, unsymmetrical t, J = 7.1 Hz, J = 6.6 Hz), 7.678 (H2', s, 68% D incorporation relative to H4' integral), 6.876 (H4', d, $J_{4,5}$ = 9.8 Hz), 5.744 (H6', d, $J_{5,6}$ = 4.5 Hz), 5.455 (H5', dd, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 4.5 Hz), 4.216 (1-NCH_3, s), 3.318 (1'-NCH_3, s).

^{1}H NMR of 5-3 10 min After the Addition of DCl. δ 9.518 (H2', s, 77% D incorporation relative to H4' intensity), 9.372 (H6 or H6', d, $J_{5,6}$ = 6.1 Hz), 9.331 (H6 or H6', d, $J_{5,6}$ = 6.2 Hz), 9.018 (H4', d, $J_{4,5}$ = 8.1 Hz), 8.854 (H4, t, $J_{3,4}$ = $J_{4,5}$ = 7.9 Hz), 8.450 (H5 or H5', unresolved dd), 8.33-8.42 (H5 or H5' and H3, m), 4.53 (1'-NCH_3, s), 4.328 (1-NCH_3, s). A trace of adduct remains visible in the baseline.

Suggested methoxide adduct of 1-methyl-1'-(2-(4-nitrophenyl)ethyl)-2,3'-bipyridinium diiodide in methanol-d_4 and elimination products

To an NMR tube was added 6.7 mg (0.011 mmol) of 5-10, TSP, and 0.50 mL of CD_3OD. 5-10 was largely insoluble.

To the sample was added 2 μL of 5.8 M (0.012 mmol) of sodium methoxide in CH_3OD. The solution turned yellow immediately. Most of the solid dissolved after 15 min.

Proton NMR spectra were recorded at four intervals: 15 min, 4 1/4 h, 25 1/2 h, and 4 days after preparing the sample. Spectra recorded initially contained broad peaks. Sharp peaks due to elimination products grew with time.

^{1}H NMR 15 min after Mixing. δ 8.64 (1 H, broad s), 8.26 (1 H, broad s, partially overlaps 8.20), 8.20 (o-NO_2 of nitrophenyl, 2 H, d, partially overlaps 8.26), 7.70 (2 H, broad s), 7.59 (m-NO_2 of nitrophenyl), 6.77 (1 H, broad s), 5.78 (1 H, broad s), 5.56 (1 H, broad s), 4.15 (NCH_3, 3 H, s, partially overlaps 4.01), 4.01 (1 H, broad s, partially overlaps 4.15), 3.79 (1 H, broad s), 3.25 (CH_2 on nitropheyl, t, partially overlaps CH_3 of CH_3OD). The signal for one proton is missing and may be under the OH or CH_3 peaks of solvent and methoxide.

^{1}H NMR 4 Days After Mixing. Shifts for 4-7: δ 9.12 (H6, d), 8.86 (H6',d), 8.1-8.3 (H4', H3, H5, m, overlap nitrostyrene), 7.74 (H5', d), 4.25 (NCH_3, s). Protons H6 and H6' are nearly completely exchanged by deuterium. H2' is completely exhanged. The N-methyl is partially exchanged and an upfield deuterium isotope shift of 0.02 ppm is observed for the $NCDH_2$ peak which is a triplet. Shifts for 4-nitrostyrene: 8.1-8.3 (ortho to NO_2, overlaps 4-7), 7.68 (meta to NO_2, d), 6.87 (α vinyl, dd), 6.03 (β vinyl, d), 5.51 (β vinyl, d). Both β vinyl protons underwent D exchange to a

small extent. An isotope shift of 0.02 ppm is observed for the β vinyl signal in the deuterio compound.

Proposed hydroxide adduct of 1'-(2-(4-nitrophenyl)ethyl)-1-methyl-2,3'-bipyridinium diiodide in DMSO-d_6 and subsequent elimination

An NMR solution contained 5.7 mg (0.0096 mmol) of 5-10 in 0.50 mL of DMSO-d_6 with TSP as an internal reference. To the solution was added 2 μL (0.013 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The sample turned red immediately on mixing. ^{1}H NMR spectra were recorded 15 min, 25 min, 90 min, and 20 h after addition of DBU. After 15 min, a mixture of 5-10, adducts, and elimination products are present. The amount of elimination products increases with time and the amounts of starting material and adducts decrease. After 20 h, the reaction contained only DBU and the expected elimination products.

Longitudinal Relaxation Times

Preparation of NMR samples

Nickel(II) chloride hexahydrate was dried for 6 to 8 hrs in a drying pistol under vacuum while heated with refluxing water. The bright green hexahydrate turned yellow on dehydration. "Ultrapure" sodium dodecylsulfate purchased from U. S. Biochemicals and D_2O (99.9%) purchased from Merck & Co. were used for solutions containing 7-1. Electrophoresis grade sodium docecylsulfate purchased from U. S. Biochemicals and D_2O (99.8%) purchased from Aldrich

Chemical Co. were used for solutions of Ado and iAdo. Ado and iAdo were reagent grade and purchased from Aldrich and Sigma Chemical Companies, respectively. Ado, iAdo, and SDS were used without further purification. A teflon coated spatula was used when weighing solids and plastic pipet tips and syringe needles were used when measuring liquids.

1-Methyl-4,4'-bipyridinium Iodide (7-1) Solutions. Three stock solutions containing 0.100 M 1-methyl-4,4'-bipyridinium iodide, 0.363 M SDS, and 79 mM $NiCl_2$ were prepared by placing the appropriate amount of solid into dried volumetric flasks and diluting to the mark with D_2O. Two NMR samples containing 30 mM 7-1 and 30 mM 7-1 with 0.18 M SDS were prepared by diluting one aliquot of the 7-1 stock solution with D_2O and another aliquot with D_2O and with SDS solution. After T_1 measurements were made, a small aliquot (10 μL to a 500 μL sample) of the Ni^{2+} stock soltuion was added to each sample and T_1 measurements were repeated. The addition of the small volume of $NiCl_2$ solution did not change the concentrations of 7-1 or SDS significantly.

Adenosine (Ado) and 2',3'-Isopropylidene Adenosine (iAdo) Solutions. Three new stock solutions were prepared containing 34 mM Ado, 0.300 M SDS, and 52 mM $NiCl_2$ in D_2O. The Ado solution was heated in a water bath to effect dissolution but recrystallized on standing for several hours. Before preparing NMR samples, the solid was redissolved. Two solutions containing 20 mM Ado and 20 mM Ado with 0.12 M SDS were prepared by diluting 300 μL aliquots of Ado with 200 μL

of D_2O and with 200 μL of SDS solution. After making T_1 measurements, 10 μL of $NiCl_2$ solution was added to the 20 mM Ado sample to make a solution containing 20 mM Ado and 1.0 mM $NiCl_2$. A new sample containing 20 mM Ado, 0.12 M SDS and 1.0 mM $NiCl_2$ was prepared by mixing 400 μL of 25 mM Ado/0.15 M SDS in D_2O, 18.5 μL of 27 mM $NiCl_2$ in D_2O and 81.5 μL of D_2O.

2',3'-Isopropylideneadenosine was weighed into two NMR tubes (3.4 and 3.5 mg) and one sample was diluted with D_2O and the other with D_2O and 0.300 M SDS in D_2O to make two separate solutions containing 20 mM iAdo and 20 mM iAdo with 0.12 M SDS. The sample containing iAdo in D_2O partially crystallized while T_1 values were being measured. After making T_1 measurements, an aliquot of 52 mM $NiCl_2$ in D_2O was added to each sample to make two new solutions, one containing 20 mM iAdo and 1.0 mM $NiCl_2$ and the other containing 20 mM iAdo, 0.12 M SDS and 1.0 mM $NiCl_2$.

A few crystals of TSP were added to each NMR sample as an internal standard. NMR samples which did not contain SDS were degassed by bubbling N_2 through a D_2O bubbler and then through the samples for 20-30 min immediately before making T_1 measurements except for the Ado/$NiCl_2$ solution which was not degassed. The samples containing SDS but no $NiCl_2$ were degassed by blowing N_2 over the top of the solutions and then sonicating for 15 s. Samples containing SDS and Ni^{2+} were not degassed as the T_1's are short and O_2 is not likely to contribute significantly to the relaxation.

Proton T_1 measurements

T_1 measurements were recorded on a Varian VXR-300 using the inversion-recovery routine available in its software but using a composite 180° pulse for inversion instead of the standard 180 ° pulse. Samples were allowed to equilibrate in the probe at 25.0 °C for at least 20 min before begining T_1 measurements. Longitudinal relaxation times were measured for all of the protons of a given sample in one experiment. For solutions of 7-1, 10 delay times (τ values) were used in each experiment which ranged in length from about 0.05 times the shortest T_1 value to 4 times the longest T_1 value. For solutions of Ado and iAdo, 9 - 12 τ values were used which ranged from about 0.06 times the shortest T_1 value to 4 times the longest T_1 value. The spectra at each delay time consisted of 32 or 64 transients. Values of T_1 were calculated using the routine on the spectrometer which computes an exponential fit of the peak intensities. Values of T_1 were calculated for each line of a multiplet and then averaged to arrive at a value for that proton signal.

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BIOGRAPHICAL SKETCH

Linda B. Bloom was born on March 28, 1964, in Alexandria, Virginia. She grew up in North Carolina and attended Charles E. Jordan High School in Durham, North Carolina, graduating in June of 1982. She received the degree of Bachelor of Science with distinction in chemistry from the University of North Carolina at Chapel Hill in May of 1986. She is a member of Alpha Chi Sigma Chemistry Fraternity as well as Phi Beta Kappa.

In August of 1986, she enrolled at the University of Florida to pursue the degree of Doctor of Philosophy in organic chemistry. She is married to David C. Bloom who received his Ph.D. in May of 1990 from Vanderbilt University. After completion of her degree, she will be doing postdoctoral research on the mechanisms of DNA polymerases with Dr. Myron Goodman and Dr. John Petruska at the University of Southern California.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John A. Zoltewicz,
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Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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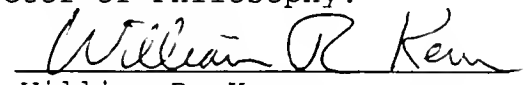

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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